PERSPECTIVES

On Hardy-Weinberg in Introductory Biology: Teaching this Fundamental Principle in an Authentic, Engaging, and Accurate Manner

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Abstract: The Hardy-Weinberg principle (HWP) is a fundamental model upon which much of the discipline of population genetics is based. Despite its significance, students often leave introductory biology courses with only a shallow understanding of the use and implications of the HWP. I contend that this deficiency in student comprehension is too-often a consequence of teachers of introductory biology having an insufficient mastery of the HWP, as well as a lack of proficiency in quantitative analysis in general. The purpose of this Perspectives paper is to correct some common misconceptions about the HWP so that teachers of introductory biology will have more confidence that they are communicating this important principle correctly to their students. A companion Innovations paper provides a problem-set of six real-world Hardy-Weinberg scenarios, along with explicit instructions on quantitatively analyzing the scenarios.

Keywords: evolutionary mechanisms, Hardy-Weinberg equilibrium, introductory biology, population genetics

The Centrality of the Hardy-Weinberg Principle in Evolutionary Biology

The Hardy-Weinberg principle (HWP) often serves as the foundation for students to build an understanding of how evolution works in populations of organisms. Nevertheless, applying the HWP tends to be one of the more difficult and thus least favorite topics for introductory biology students, due in part to the abstract (probabilistic) nature of the HWP, students' preconceptions that biology should not include mathematics, and students' lack of appreciation of the variety of real-world biological scenarios to which the HWP can be applied (Mertens, 1992; Winterer, 2001; Brewer & Gardner, 2013; Smith & Baldwin, 2015). It does not help that instructors of introductory biology are often not specialists in population biology and may be just as uncomfortable with the quantitative aspects of HW problems as their students are (Masel, 2012). As a result, the teaching of the HWP tends to be superficial, and students often move on to higher-level biology courses with no deeper knowledge of the HWP than remembering a partial list of assumptions and the formula "$p^2 + 2pq + q^2 = 1$." Worse yet, biology departments may decide to remove the teaching of HW from introductory courses altogether (Masel, 2012).

I contend that it is a disservice to students to give superficial treatment to the HWP in an introductory biology course for several reasons. First, this topic serves as an entrée into the increasingly important discipline of population genetics. Failure to do it justice may result in a missed opportunity to attract or retain students whose interest in biology tends more to the macro-scale than the cellular and molecular perspectives that constitute much of the course matter in introductory biology. Second, the HW perspective provides students with an appreciation that evolution is a phenomenon that happens continuously and that can be studied in real time (Winterer, 2001). Third, using the HWP to analyze real (or creatively contrived) data provides an excellent way for students to employ the scientific method, including using simple statistics to test hypotheses. Thus, analysis of evolutionary scenarios using the HWP provides teachers an opportunity to answer the pedagogical call to incorporate quantitative activities into lecture courses (NRC, 2003; AAAS, 2011; Hoskinson et al., 2013; AAAS, 2015; McLaughlin & Metz, 2016). Fourth, solving problems where the HWP is applied in an authentic way (not just plugging values into a formula) requires students to use higher-order cognitive processes (e.g., analysis and evaluation in Bloom's taxonomy) (Crowe et al., 2008).

The Hardy-Weinberg Equilibrium

A central question throughout the history of the study of evolutionary biology is why genetic variation persists within populations. The work of Gregor Mendel, which was rediscovered by the scientific community in 1900, put to rest the widely held belief in blending inheritance in favor of particulate inheritance, in which phenotypes are expressed in either a dominant or recessive form. Still, many biologists reasoned that populations ought to evolve to the point where dominant phenotypes completely replace other phenotypes, thus eliminating genetic variation within populations.

Within a decade of the rediscovery of Mendel's work, the English mathematician Godfrey Hardy and the German physician Wilhelm Weinberg demonstrated analytically how and why populations maintain more than one allele for many genes, rather than one allele always spreading to fixation (Hardy, 1908; Weinberg, 1908; Raven et al., 2014). Hardy and Weinberg demonstrated that, for a gene with two alleles (in a sexually reproducing population of diploid individuals), the proportion of individuals of different genotypes will tend to stabilize at specific frequencies that are determined solely by the frequencies of the alleles in the population—a model now referred to as the "Hardy-Weinberg principle."

The HWP is often seen as consisting of two main components (i.e., implications or conclusions) (Hartl & Clark, 1989; Freeman & Herron, 2004; Masel, 2012). The first is that the allele frequencies for a gene will remain constant across generations within a population—as long as certain conditions are met. To illustrate, consider a gene with two alleles ("A" and "a") at respective frequencies of p and q in a population. These frequencies will remain at p and q as long as no force is acting to change them. Because "evolution" is often defined as a change in allele frequencies in a population over time, and these forces of change are considered "evolutionary mechanisms," this first implication is at risk of being stated in a circular fashion (e.g., "Evolution requires the action of evolutionary mechanisms") that belies the novelty of this conceptual insight.

The second main component of the HWP is perhaps of more practical use. It states that the frequencies at which the genotypes will stabilize are p^2 for AA, q^2 for aa, and $2pq$ for Aa. These genotype frequencies are referred to as the "Hardy-Weinberg equilibrium" (HWE) frequencies, and a departure from these frequencies can be seen as evidence of the action of one or more evolutionary mechanisms. Note that the first component of the HWP has to do with allele frequencies across generations, while the second component has to do with genotype frequencies within a generation.

Why Populations may Deviate from Hardy-Weinberg Equilibrium

Mutation

There are several biological phenomena that can cause a population to depart from HWE for a gene. Most simply, a gene-copying error can cause an allele to change into a different allele in an individual (e.g., "A" mutates into "a"). Such a mutation instantly changes the allele frequencies, and thus the genotype frequencies, within the population. However, mutations are so infrequent that the changes in allele and genotype frequencies they cause in a single generation are generally not statistically detectable. Thus, by themselves, mutations are not likely to lead to substantial deviations of genotype frequencies from HWE. Nevertheless, mutations are essential for

the creation of genetic variation within populations, which is the raw material on which evolution acts.

Gene Flow

The HWP is strictly meant to apply to closed populations. The introduction of alleles caused by emigration of individuals from a population, immigration of individuals into a population, or fertilization of gametes by gametes that have arrived from other populations (e.g., in airborne pollen) can cause a change in allele frequencies for one or more genes in a population. These phenomena of mixing of alleles between populations—commonly called "gene flow"—can result in genotype frequencies that differ from HWE.

Non-random Mating

The HWP is also only meant to apply to populations in which alleles for a gene recombine randomly during sexual reproduction. This random mixing will occur only if individuals in the population do not preferentially mate with individuals that share their genotype (i.e., assortative mating) or preferentially with individuals that have a different genotype than their own (i.e., disassortative mating). Although non-random mating within a population will not change the allele frequencies, it can cause the genotype frequencies for one or more genes related to mating preferences to differ substantially from HWE frequencies. Specifically, assortative mating will cause an excess of homozygotes, and disassortative mating will cause an excess of heterozygotes, relative to HWE frequencies for a given gene.

Natural Selection

If individuals of different genotypes in a population produce different numbers of offspring (i.e., genotypes vary in fitness), allele frequencies will change from one generation to the next, and genotype frequencies will differ from HWE, for any gene that is correlated with fitness. Specifically, a genotype with higher-than-average relative fitness will occur at a higher frequency than would be predicted by the HWE, while a genotype with lower-than-average relative fitness will occur at a lower frequency than the HWE prediction. These differences in fitness among genotypes—whether caused by natural, sexual, or artificial selection—are the most important causes of deviations from HWE in terms of driving adaptive evolution within a population. In practical terms, however, deviations from HWE caused by natural selection tend to be relatively small compared to deviations caused by non-random mating (Hartl & Clark, 1989; Masel, 2012). Therefore, the intensity of the selection must be rather high, or the sample very large, in order to infer that deviations from HWE frequencies due to natural selection are statistically significant.

Genetic Drift

Finally, the genotype frequencies for any given gene will rarely exactly match the HWE frequencies

due to random chance alone—a phenomenon most commonly referred to as "genetic drift." The unpredictability of such departures from HWE frequencies may be intuitively understood by students, as it can be related to concrete probability examples such as the flipping of coins. For instance, if a coin is flipped 100 times, students would not be surprised if it landed on "heads" 52 times or 46 times. Analogously, students have little difficulty with the notion that genotypic frequencies can differ slightly from HWE just due to sampling "noise." Nevertheless, students' misunderstandings of the subtleties involved in genetic drift, and thus overall importance of genetic drift as a mechanism of evolution, belie the seeming simplicity of the concept of noise. In fact, even advanced students tend to harbor misconceptions about the causes or significance of genetic drift (Andrews et al., 2012; Price et al., 2014).

Genetic drift has probabilistic, stochastic, essentially random effects on allele and genotype frequencies. Statisticians would equate such effects with the phenomenon of sampling error. While such technical terms may be clear to experts, their esoteric and nuanced meanings can be a major source of confusion for students (Masel, 2012; Wang, 2016). As a result, students may come to miscomprehend genetic drift as merely a statistical artifact or an error in sampling technique, rather than a major driver of evolution in natural populations. In addition, they may lose sight of the fact that genetic drift is caused by actual biological processes—the random joining of gametes and chance events in the lives of individuals that may affect their survival or reproductive success (Price et al., 2014).

 Genetic drift differs from the other major evolutionary mechanisms mentioned above because it is constantly occurring in every population of every species. Thus, changes in allele frequencies due to genetic drift are inevitable. In some generations, the effect of genetic drift on allele and genotype frequencies may be quite small, while in others, the effects may be dramatic. For instance the allele frequencies can change drastically within a single generation in a population whose size is severely reduced due to a natural disaster, or in a new population that is established by a small number of individuals—phenomena respectively referred to as a "genetic bottleneck" and a "founder effect." These phenomena highlight the fact that the relative effects of genetic drift are greater the smaller a population is, even though random, stochastic changes in allele and genotype frequencies occur in small and large populations alike.

The HW Equilibrium as a Null Hypothesis to Test for Evolution

Although Hardy and Weinberg merely aimed to demonstrate how multiple alleles could be maintained for genes in natural populations of organisms, their principle became one of the most useful tools of evolutionary biologists. The great power of the HWP is as a null hypothesis to test whether a population is undergoing evolution at any particular gene of interest. Specifically, if the genotype frequencies depart from the HWE expectations within a generation, then we can infer that the population is undergoing evolution. The magnitude and direction of the departure can inform which mechanism of evolution may be occurring (e.g., selection, immigration, or assortative mating), and the inferences about evolutionary mechanisms can be supported by information on the ecological setting in which the population is living.

The relatively simple arithmetic involved in working with the HWP has helped to make "Hardy-Weinberg problems" a staple of introductory biology courses, even in secondary schools. However, this simplicity in mathematical calculations belies the nuance and sophistication required in creating and accurately interpreting HW problems that involve authentic evolutionary scenarios.

What is Wrong with the Typical HW Problems Presented to Students?

Below, I focus on two categories of shortcomings that I have most commonly observed in HW problems that are written for students in introductory biology courses. The first has to do with the simplicity, banality, and lack of intellectual engagement required by the problems. The second shortcoming results from a conflation of the two main components of the HWP, resulting in a misunderstanding of the time-scale on which the HWE genotype frequencies are meant to apply.

Superficiality of Problems

Despite the rich potential for examining evolutionary mechanisms, most of the HW problems presented to students treat the HW principle in simplistic, superficial manner (Smith & Baldwin, 2015). Specifically, students are asked to solve problems in which they are given frequencies of phenotypes and/or alleles (usually % of homozygous recessive individuals) in a population and asked to calculate the genotype frequencies, given that all assumptions of the HW principle are met. Thus, they are taught merely to plug values into the HW equations: $p + q = 1$ and $p^2 + 2pq + q^2 = 1$. The solution of such problems entails the employment of only lower- to mid-level cognitive skills, such as memorization and application (Crowe et al., 2008). Moreover, this approach promotes a false impression about evolution in natural populations. That is, focusing on problems in which populations are assumed to be in HWE reinforces the notion that populations in nature are always in HWE. Not only is this notion false, these practice problems ignore the truly interesting aspect of the HWE: that departures from HWE indicate evolution in action!

A better pedagogical strategy for posing Hardy-Weinberg problems is to introduce scenarios in which the HWE frequencies are violated (McMurran, 2010). Enough information on numbers of individuals with particular phenotypes or genotypes must be provided so that students can calculate the actual allele and genotype frequencies they will need to address the question. The question should require them to compare the actual data to the HWE expectations to infer whether they differ. If they differ, then students can propose explanations as to what evolutionary mechanism is most likely to be causing the difference. Some information on the natural history of the organisms and the ecological setting experienced by the population should be given in the text of the problem to provide sufficient hints for the students to generate explanations. For examples of these sorts of scenarios, refer to the companion Innovations paper on Hardy-Weinberg problems in this issue. That paper contains six real-world problems with detailed solutions (including chi-square tests) that can be used as-is or easily adapted to suit an instructor's particular goals.

Time-Scale Issue

The suggestion to provide students with more realistic scenarios to which they can apply the HW principle to make inferences about evolution is certainly not novel. However, many well-intentioned teachers write problems that perpetuate the misconception that the HWE frequencies can be applied to genotypic data *across* generations (Smith & Baldwin, 2015). In reality, the HWE genotype ratios are meant to apply to allele and genotype frequencies *within* a generation. That is, the allele frequencies from one generation cannot be validly used to calculate HWE genotype frequencies that are then compared to observed genotypic frequencies in a subsequent generation—though students are often instructed to do just that (McMurran, 2010).

This mistake of applying the HWE across generations seems to arise from teachers focusing all their efforts on coming up with evolutionary scenarios that violate one of the assumptions of the HWP (viz., no mutations, no natural selection, no gene flow, random mating, and "infinite" population size), but not considering whether applying the HWE frequencies is even the best way to analyze the evolutionary scenario they created.

A scenario in a typical "HW" problem either provides (or requires students to calculate) allele, genotype, and/or phenotype frequencies for a gene of interest over a series of generations. To test whether evolution occurred in the population, students need to assess whether there has been a change in the allele frequencies in the population over time. A straightforward way to address this question would be a chi-square test of whether the relative numbers of individuals of the various genotypes (or phenotypes) in the first generation are significantly different from the relative numbers of individuals of the same genotypes (or phenotypes) in the final generation. If the chi-square value is statistically significant (i.e., $P < 0.05$), then students can infer that evolution has occurred for the gene of interest. Clues from the set-up of the problem can be used to hypothesize which mechanism of evolution (e.g. natural selection, gene flow, or genetic drift) was most likely to have caused evolution in this scenario.

Note that no application of the HWE genotype frequencies was necessary for the evolutionary analysis described in the previous paragraph. However, trouble often arises when students are required to apply the HWE frequencies to this sort of scenario (in which allele and genotype frequencies are available across generations). Specifically, students are often instructed to calculate HWE genotype frequencies from initial allele frequencies, then compare these equilibrium frequencies to the final genotype frequencies. If the final observed genotype frequencies differ from the initial HWE frequencies, then students infer that evolution has occurred. This sort of analysis may sound reasonable, and it would seem to fulfill the teacher's goal of teaching the HWE frequencies in the context of analyzing evolution. However, this analysis violates a fundamental aspect of the HWP: it uses allele frequencies from one generation to calculate HWE expectations for a different generation (which may well have different allele frequencies).

Despite this common mistake, it is possible for students to employ the HWE frequencies in a valid fashion to this scenario to test for evidence of evolution. Specifically, they could use the actual allele frequencies from the last generation to calculate HWE genotype frequencies, then compare these HWE frequencies to the observed genotype frequencies in the last generation. In fact, it would be valid to use the allele and genotype frequencies *within* any of the generations to test for evidence of evolution in this scenario. The inclusion of multiple generations of frequencies is actually superfluous if the sole goal is for students to apply the HWE frequencies to test for evolution.

These two approaches to looking for evidence of evolution (that is, by analyzing changes in allele frequencies across generations and by analyzing departures from HWE genotype frequencies within a generation) can be seen as complementary strategies, each with their own strengths. For instance, if the fitness differences across phenotypes are slight, then departures of actual genotype frequencies from HWE genotype frequencies may be too small to detect the action of evolution. However, the change in allele frequencies after many generations of individuals experiencing the same intensity of selection may be quite appreciable (and statistically significant).

In other cases, comparing the actual genotype frequencies with the HWE frequencies may indicate

that natural selection is acting within generations, even if allele frequencies do not change across generations in the population. For instance, consider a gene with two alleles ("B" and "b") for which both types of homozygotes (BB and bb) are equally common, have equal fitness, and mate randomly with respect to genotype. Heterozygotes (Bb) also mate randomly, but they reproduce at only half the rate as the homozygotes. The allele frequencies in this population may remain the same from generation to generation (i.e., $p = q = 0.5$), but the population will never be at HWE because there will always be an excess in the proportion of both homozygotes and a deficiency in the proportion of heterozygotes.

CONCLUSION

In this Perspectives article, I have identified HW-related topics where students (and teachers) are likely to harbor conceptual misunderstandings. (In a companion Innovations article, I illustrate these misconceptions with empirical scenarios and highlight common computational mistakes.) Awareness of these problem areas should help provide teachers with the tools required to teach the Hardy-Weinberg with confidence, and thus instill among their students a better appreciation for how evolution works at the population-genetics level. With the knowledge that evolution can be observed and measured in contemporary populations, introductory-biology students may be inspired to pursue the study of evolution in higher-level courses in the biology curriculum.

REFERENCES

AAAS. 2011. *Vision and Change in Undergraduate Biology Education: A Call to Action*. American Association for the Advancement of Science, Washington, D.C. 79p.

AAAS. 2015. *Vision and Change in Undergraduate Biology Education: Chronicling Change, Inspiring the Future*. American Association for the Advancement of Science, Washington, D.C. 58p.

ANDREWS, T.M., R.M. PRICE, L.S. MEAD, T.L. MCELHINNY, A. THANUKOS, et al. 2012. Biology undergraduates' misconceptions about genetic drift. *CBE-Life Sciences Education* 11: 248-259.

BREWER, M.S., AND G.E. GARDNER. 2013. Teaching evolution through the Hardy-Weinberg principle: a real-time, active learning exercise using classroom response devices. *The American Biology Teacher* 75(7): 476-479.

COUNCIL, N.R. 2003. *BIO 2010: Transforming Undergraduate Education for Future Research Biologists*. The National Academies Press, Washington, D.C. 191p.

CROWE, A., C. DIRKS, AND M.P. WENDEROTH. 2008. Biology in bloom: implementing Bloom's taxonomy to enhance student learning in biology. *CBE-Life Sciences Education* 7(winter): 368-381.

FREEMAN, S., AND J.C. HERRON. 2004. *Evolutionary Analysis*. *third ed*. Pearson Education, Inc., Upper Saddle River, NJ. 802p.

HARDY, G.H. 1908. Mendelian proportions in a mixed population. *Science* 28(706): 49-50.

HARTL, D.L., AND A.G. CLARK. 1989. *Principles of Population Genetics*. *second ed*. Sinauer Associates, Inc., Sunderland, MA. 682p.

HOSKINSON, A.-M., M.D. CABALLERO, AND J.K. KNIGHT. 2013. How can we improve problem solving in undergraduate biology? Applying lessons from 30 years of physics education research. *CBE-Life Sciences Education* 12: 153-161.

MASEL, J. 2012. Rethinking Hardy-Weinberg and genetic drift in undergraduate biology. *Bioessays* 34: 701-710.

MCLAUGHLIN, J., AND A. METZ. 2016. Vision & Change: why it matters. *Teachers College Record* 78(6): 456-462.

MCMURRAN, S.L. 2010. The Hardy-Weinberg principle. *PRIMUS* 20(6): 529-549.

MERTENS, T.R. 1992. Introducing students to population genetics & the Hardy-Weinberg principle. *The American Biology Teacher* 54(2): 103-107.

PRICE, R.M., T.C. ANDREWS, T.L. MCELHINNY, L.S. MEAD, J.K. ABRAHAM, et al. 2014. The genetic drift inventory: a tool for measuring what advanced undergraduates have mastered about genetic drift. *CBE-Life Sciences Education* 13: 65-75.

RAVEN, P.H., G.B. JOHNSON, K.A. MASON, J.B. LOSOS, AND S.R. SINGER. 2014. *Biology*. *tenth ed*. McGraw-Hill, New York. 1279p.

SMITH, M.U., AND J.T. BALDWIN. 2015. Making sense of Hardy-Weinberg equilibrium. *The American Biology Teacher* 77(8): 577-582.

WANG, C. 2016. Exploration of the concept of genetic drift in genetics teaching of undergraduates. *Hereditas (Beijing)* 38(1): 82-89.

WEINBERG, W. 1908. Über den Nachweis der Vererbung beim Menschen. *Jahreshefte Vereins vaterländische Naturkunde in Württemberg* 64: 369-382.

WINTERER, J. 2001. Hardy-Weinberg equilibrium and evolution. *The American Biology Teacher* 63(9): 678-687.