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Balancing Selection: Walking a Tightrope

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Combining modern transgenic techniques with fitness measurements and enzyme activity assays, a new study demonstrates a habitat-dependent tradeoff between two alleles of a key detoxification enzyme in fruit flies. The elegant findings provide concrete, elusive evidence supporting a foundational and controversial theory about the maintenance of genetic variation.

Genetic variation is a ubiquitous property of natural populations, and its maintenance in the face of random and deterministic forces is at the heart of one of the great debates in evolutionary biology. This variation arises from new mutations, changes in DNA sequences spanning single-nucleotide polymorphisms to whole genome duplication events, and is the substrate for evolutionary change. Such mutations can be advantageous, neutral or deleterious — a range prefigured by Charles Darwin who pondered the fate of "favourable", "injurious" and "neither useful nor injurious" variations as he outlined the process of evolution by natural selection [1]. Population genomics has now revealed that genomes of a randomly chosen pair of individuals from the same species generally differ by 0.1% (for example, in humans) to 10% of their sequence [2]. Such findings have helped energize the debate over the importance of various mechanisms that could facilitate the maintenance of such tremendous genetic variation within species. In an elegant new chapter to this debate, Chakraborty and Fry in this issue of *Current Biology* [3] demonstrate that natural selection likely acts to maintain a single amino acid polymorphism in a key enzyme used by flies to detoxify dietary ethanol byproducts. Leveraging modern genetic tools, including insertion of alternative alleles of this enzyme into the genomes of isogenic flies, coupled with enzymology and laboratory fitness studies, their study sets a new bar in the field.

To place Chakraborty and Fry's study in context, a history of the field is helpful (Figure 1). In the mid-1900s, as methods emerged to observe genetic variation directly, interest in explaining patterns of genetic variation within natural populations surged. Decades before DNA sequencing, Dobzhansky and colleagues peered through microscopes at dye-stained chromosomes, cataloguing variation in the orientation of large stretches of DNA in fruit flies (*Drosophila* species) [4]. They proposed that this variation persisted through the action of balancing selection, a collective term for evolutionary processes that adaptively maintain variation in populations. Specifically, they hypothesized that fruit from different plant species provided spatially distinct habitats exerting different selection pressures on flies, and genetic variation persisted because no one chromosomal variant was superior across all habitats. Levene confirmed mathematically that Dobzhansky's intuition could occur [5]. Dempster then showed that selection pressures varying in time, rather than space, could also maintain genetic variation [6]. Over the ensuing decades, as dozens of expansions of these models were constructed [7] — including models for traits controlled by many genes [8], in contrast to Levene's single locus model — empirical evidence for balancing selection also began to mount (e.g., [9]).

In the 1960s, Hubby and Lewontin captivated evolutionary biologists when they uncovered surprisingly high levels of genetic variation in *Drosophila* allozymes [10]. Balancing selection, and spatially varying selection in particular, became a popular explanation for the maintenance of this variation. By 1974, merging theory with natural observations, Gillespie and Langley proposed that spatially varying selection might be the primary evolutionary process responsible [11].

Alternative explanations, however, tempered the enthusiasm for widespread balancing selection in nature. Kimura's neutral theory of molecular evolution, now

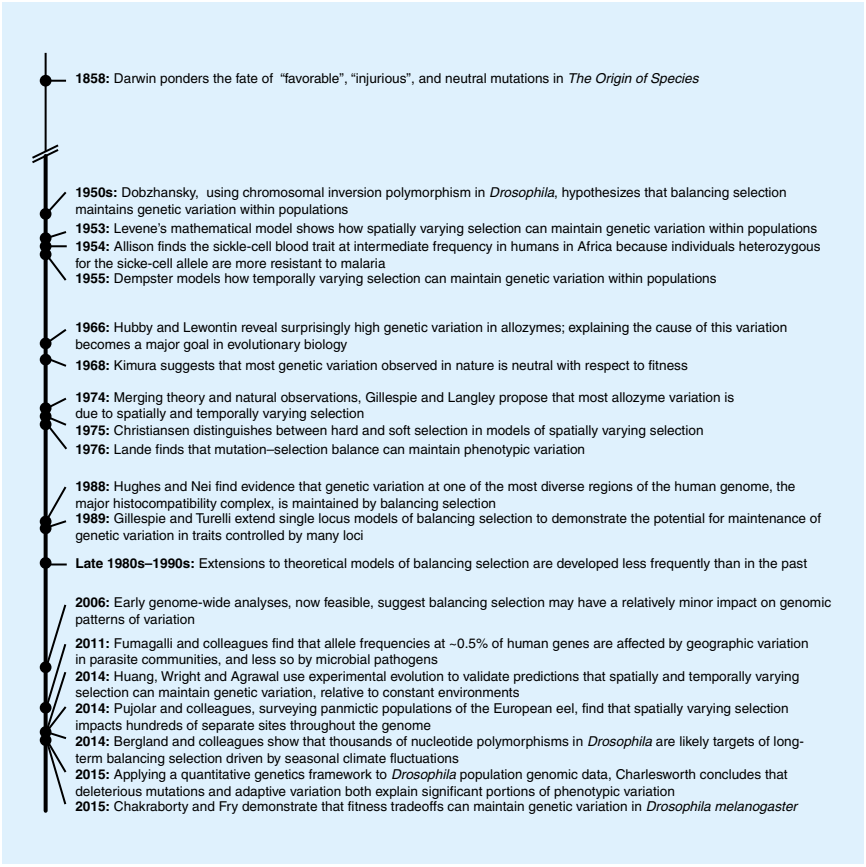

Figure 1. Timeline of some major theoretical and empirical advances in the debate over balancing selection's role shaping patterns of genetic variation (citations are provided in the main text.)

widely accepted (with some modification) and used as a null model in population genetics, posited that most genetic variation is neutral with respect to fitness [12]. These neutral variants, introduced by mutation, could rise to high frequency stochastically. But the possibility still remained that *functional* genetic variation — the variation that affects organismal phenotypes — might largely be maintained by balancing selection. However, Lande showed balancing selection need not be invoked to explain functional genetic variation [13]. Instead, in contrast to the adaptive functional genetic variation envisioned by Dobzhansky, the input of deleterious variants by mutation and their purging by natural selection could result in the persistence of deleterious genetic variation. Even extensions of Levene's model became more restrictive [14]. By the late 1980s, new extensions to Levene's model became relatively infrequent [7], perhaps reflective of skepticism for widespread balancing selection as a major cause of genetic variation. Early genomic analyses in the mid-2000s led experts to conclude that spatially varying selection might contribute rather little to the abundance of genetic variation within natural populations [7].

The past few years, however, have seen the development of powerful methods to detect balancing selection and, importantly, enormous genomic datasets to apply them. The result has been a resurgence of evidence pointing to widespread balancing selection in nature. Foreshadowed by Hughes and Nei's discovery that balancing selection generated the most pronounced hotspot of genetic diversity in the human genome [15], whole genome analyses by Fumagalli and colleagues found that geographically variable selection pressure from parasites causes allele frequency differences between populations in 0.5% of human genes [16].

Unsurprisingly, *Drosophila* remain the subject of some of the most elegant studies revealing widespread balancing selection. Nucleotide polymorphisms at thousands of sites across fly genomes may be maintained by seasonal oscillations in climate [17], and experimental evolution coupled with genome sequencing supports the prediction that populations experiencing spatial or temporal variation in selection pressure are more genetically diverse than those in constant environments [18]. Applying quantitative genetic theory to new genomic data from *Drosophila*, Brian Charlesworth recently concluded that while much genetic variation affecting fitness is deleterious, balancing selection must be invoked to explain the persistence of a substantial portion [19]. Beyond *Drosophila*, evidence for widespread balancing selection is emerging from non-traditional model systems, such as the detection of loci under spatially varying selection in the panmictic European eel [20].

While genomic studies now suggest balancing selection might be widespread, few studies have drawn the link between single genetic polymorphisms, the resulting variation in biochemical and organismal phenotypes, their effects on fitness, and the environmental pressures that act to maintain such variation. In the new study [3], Chakraborty and Fry leverage modern genetic techniques to elucidate these links. Fittingly, they investigate genetic variation in a detoxification enzyme potentially maintained by the same spatially varying habitat that once captivated Dobzhansky: the range of different fruit used by *Drosophila* as feeding substrates. Specifically, they focus on the effect of variation in ethanol concentration, which ranges from greater than 5% to nearly 0% in rotting fruit. Aldehyde dehydrogenase in *D. melanogaster* has two functions: detoxifying acetaldehyde produced from ethanol consumed during feeding and detoxifying aldehydes produced via oxidative phosphorylation regardless of diet. They hypothesized that two alleles, differing at a single amino acid, were

maintained by tradeoffs in enzyme substrate specificity. *In vitro* enzymology assays revealed that proteins encoded by the two alleles functioned as predicted: the enzyme encoded by the derived allele — most common in regions where *Drosophila* feed on fruit with high ethanol concentrations — increased the turnover rate of acetaldehyde but decreased it for larger aldehydes. Then, they created two experimental habitats approximating fruit with high (>5%) and low (0%) ethanol concentrations experienced by *Drosophila* in nature. *In vivo* allele swaps revealed that the derived allele increases lifetime fitness on media with high ethanol concentrations, but decreases fitness in the absence of ethanol (Figure 2).

The study by Chakraborty and Fry reflects the high emerging standard for functional studies of loci putatively under balancing selection. The work is integrative — including examination of allele frequencies in natural populations, measurements of fitness in transgenic flies differing only by the polymorphism of interest, and assays of enzyme function — with resolution down to a single nucleotide change. However, definitively showing the links between genotype, phenotype, and fitness is difficult, and not yet complete. Fitness trade-offs across environments do not necessarily guarantee the long-term maintenance of polymorphisms [7]. Whether an equilibrium allele frequency exists depends not just on the strength of fitness tradeoffs among alleles across environments, but also on the proportion of individuals distributed into each environment and the amount of gene flow between environments. Knowledge of the latter is necessary to fully apply the rich body of theory on spatially varying selection, such as Levene's model (Figure 2). Further, other forms of balancing selection that may be operating, such as heterozygote advantage, remain unexplored. And the question remains: is aldehyde dehydrogenase one of many genome-wide polymorphisms potentially maintained by variation in ethanol across habitats, or simply a rare case?

What's next? As genome sequencing and *in vivo* genome editing become

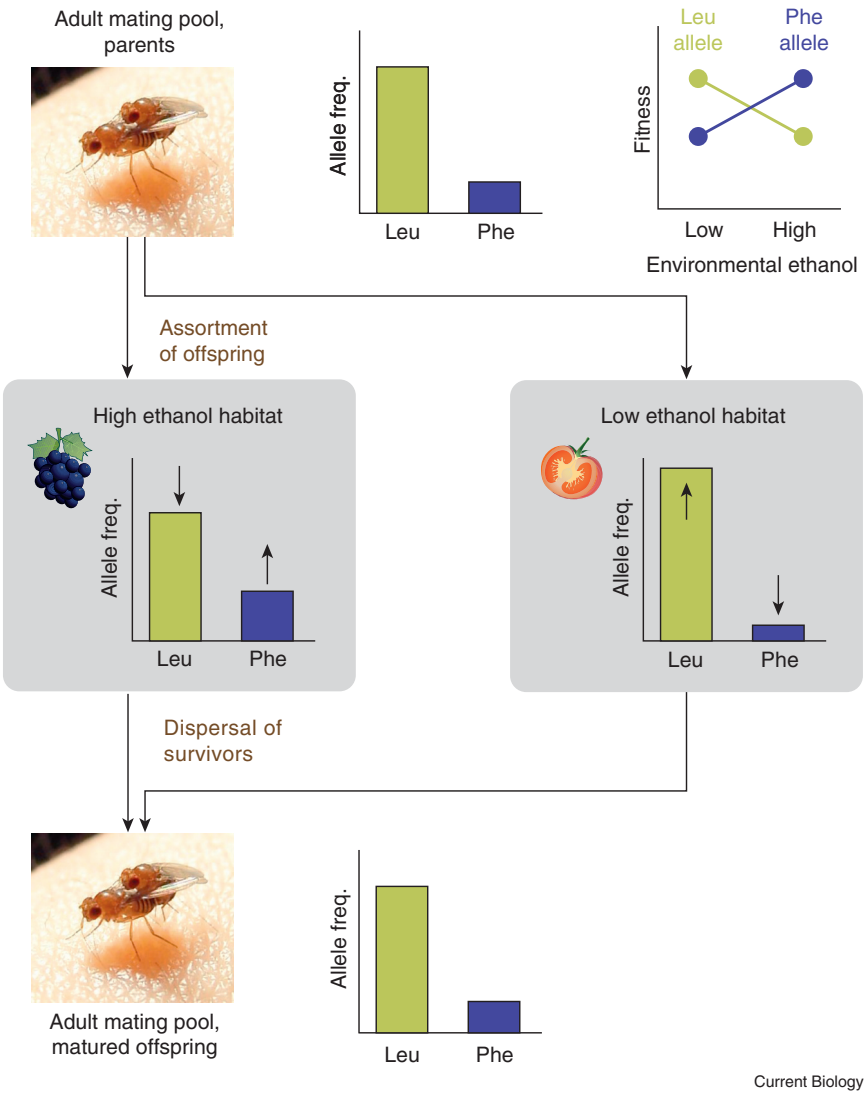

Figure 2. The maintenance of genetic variation by balancing selection.
Diagram of Levene's (1953) model of spatially varying selection applied to *Drosophila melanogaster* aldehyde dehydrogenase. Habitat-specific fitness tradeoffs are depicted for the two alleles in the direction found by Chakraborty and Fry [3]. Although allele frequencies among offspring change within habitats each generation, fitness measurements from Chakraborty and Fry suggest they could remain at a stable intermediate frequency in the population as a whole. (*Drosophila melanogaster* photos: Wikimedia Commons.)

feasible in many species, the time is ripe to apply both in-depth functional studies (like that of Chakraborty and Fry) and population genomic analyses to other organisms suited for detecting variation maintained by spatially varying selection. Herbivorous insects, as suggested by Levene, could be a particularly powerful model system. Habitats (host plants) are clearly delimited and finely interspersed, potentially enabling high gene flow as in Levene's model.

Leveraging the tremendous power of *Drosophila* as a genetic model system, Chakraborty and Fry went where Dobzhansky, an avid experimentalist, only could have dreamed. Continuing advances in genome sequencing and editing techniques, high throughput phenotyping, experimental evolution, and the integration of quantitative and population genetics could someday extend in-depth studies like Chakraborty and Fry's to genome-wide scales. For now, though, the debate over the effect of

balancing selection on patterns of genetic variation will surely continue.

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Animal Behaviour: Friendship Enhances Trust in Chimpanzees

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Individuals that participate in exchanges with delayed rewards can be exploited if their partners don't reciprocate. In humans, friendships are built on trust, and trust enhances cooperation. New evidence suggests that close social bonds also enhance trust in chimpanzees.

Ronald Reagan was responsible for popularizing a Russian proverb "trust, but verify". This proverb captures the dilemma that confronts individuals (or countries) when they venture into cooperative agreements, but are uncertain about the intentions of their partners. If there is some risk of being exploited, each side will need some guarantee that their partners will behave as promised. Formal institutions, like contracts, treaties, and international monitoring agencies, serve this purpose. But in informal interactions, we rely on knowledge of our partners' past behavior and reputation to determine who we can trust. Trust is a key element of close social bonds, like friendship [1], and friendship enhances cooperation [2–4]. In this issue of *Current Biology*, Engelmann and Hermann [5] provide evidence that close social bonds may function in a similar way in chimpanzees.

Economists define trust as an expectation about future cooperation in contexts in which there is some incentive for partners to cheat [6]. This definition is operationalized in the trust game [7]. In this game, two players are given endowments: Player 1 can send any amount of her endowment to Player 2; the experimenter will triple the allocation, and the full amount will be delivered to Player 2. Then, Player 2 is given the opportunity to make an allocation to Player 1. To avoid the possibility that subjects will be influenced by concerns about their own reputation or future benefits, strangers are paired in anonymous one-shot games. If Player 1 thinks that Player 2 will treat her fairly, then it is best to send Player 2 the whole endowment; however, if Player 1 expects Player 2 to be selfish and keep all the money, then it is best to send nothing. The majority of people who take the role of Player 2 do send back money, and the amount that they send is proportional to the amount that they have received [8].

Engelmann *et al.* [9] developed an alternative version of the trust game for chimpanzees, who cannot multiply and do not tolerate strangers at close

