Trends in
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Review

Fluctuating selection and the determinants of genetic variation

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Recent studies of cosmopolitan *Drosophila* populations have found hundreds to thousands of genetic loci with seasonally fluctuating allele frequencies, bringing temporally fluctuating selection to the forefront of the historical debate surrounding the maintenance of genetic variation in natural populations. Numerous mechanisms have been explored in this longstanding area of research, but these exciting empirical findings have prompted several recent theoretical and experimental studies that seek to better understand the drivers, dynamics, and genome-wide influence of fluctuating selection. In this review, we evaluate the latest evidence for multilocus fluctuating selection in *Drosophila* and other taxa, highlighting the role of potential genetic and ecological mechanisms in maintaining these loci and their impacts on neutral genetic variation.

Highlights

Recent observations of seasonally fluctuating allele frequencies at hundreds to thousands of loci across the genome in cosmopolitan *Drosophila* populations have highlighted fluctuating selection as a potentially important factor in the maintenance of genetic variation in natural populations.

Empirical evidence also suggests that fluctuating selection may influence genetic diversity across large portions of the genome through linkage.

Several single and multilocus theoretical treatments have been presented for fluctuating selection over the past decades, but these models have not been evaluated in light of these recent empirical findings.

We highlight evidence of fluctuating selection in species beyond *Drosophila* and explore potential causal genetic and ecological mechanisms.

The combined evidence suggests that fluctuating selection will need to be incorporated in future population genetic models that seek to understand variation in genetic diversity across species.

The maintenance of genetic variation

Determining the mechanisms responsible for maintaining genetic variation in natural populations has been a central goal of evolutionary biologists for more than a century [1]. In the past decade, the availability of population genomic datasets for numerous species has allowed this question to be directly evaluated in natural populations, reigniting historical debates regarding the role of **genetic drift** (see Glossary) and natural selection as the predominant evolutionary mechanisms shaping population genetic diversity (Box 1). Among the more intriguing findings has been the discovery that hundreds to thousands of polymorphic loci in cosmopolitan *Drosophila melanogaster* populations appear to be maintained by selection pressures that oscillate across seasons [2,3]. This has revived interest in the contribution of **fluctuating selection** to the maintenance of genetic variation within populations and the genetic and ecological factors that underlie it [4–7]. Here we examine recent empirical evidence for multilocus temporally fluctuating selection in natural and experimental populations and evaluate a range of theoretical treatments to provide an overview of recent developments in this area while highlighting the importance of these findings for the age-old question, what maintains genetic variation in natural populations?

Evidence of seasonally fluctuating selection in natural populations

Early field studies of fluctuating selection utilized phenotype observations as proxies for changes in underlying gene frequencies [8]. For instance, Fisher and Ford's classic 1947 study showed that different morphs in a natural population of the scarlet tiger moth, *Panaxia dominula*, were 'affected by selective action varying from time to time in direction and intensity, and of sufficient magnitude to cause fluctuating variation in all gene-ratios' to an extent that could not be attributed to genetic drift [9]. The development of genomic sequencing technologies in the past few decades has facilitated the direct discovery of fluctuating selection at the molecular level, with studies having used allele frequency time-series data to identify fluctuating selection acting at a single locus [10] and multiple loci [2,3,11–13] in natural populations.

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Box 1. Evolutionary causes of population genetic diversity

The first empirical evaluations of population genetic variation in 1966 used **allozyme** assays in fruit flies [49] and humans [78] to reveal surprisingly high levels of segregating genetic variation in both species, eventually prompting the development of the Neutral Theory of Molecular Evolution [79,80]. The Neutral Theory asserts that newly arising beneficial mutations are exceedingly rare relative to neutral variants, such that levels of standing genetic variation are predominantly shaped by genetic drift rather than positive selection. Later revisions of the Neutral Theory argued that the key determinant of neutrality is the strength of selection acting on a variant relative to the effective population size [81], thereby broadening the role of genetic drift to include weakly selected mutations.

An alternative to the Neutral Theory states that levels of genetic diversity can be largely explained by the action of selection on functional variants and its effects on neutral variation linked to the selected sites [64,82–84]. Such models typically consider positive and background selection [57,84] but also balancing selection [85]. Balancing selection is a form of natural selection that maintains allelic diversity at selected loci across generations [60]. Distinct mechanisms for balancing selection include **heterozygote advantage**, **sexually antagonistic selection**, fluctuating selection, and **frequency-dependent selection** [86]. Notably, while many forms of balancing selection result in polymorphisms being maintained at stable equilibrium frequencies [87], both fluctuating and frequency-dependent selection can result in oscillating allele frequencies. To distinguish between these two modes of selection, we note that fluctuating selection is mediated by an alternating environmental variable, such as temperature, resource availability, predator abundance, or population size [88], whereas frequency-dependent selection is conditional on the allele frequency (i.e., the selective value of an allele is causally dependent on its relative abundance rather than the environment).

Population genomic studies have inferred balancing selection in several species, including humans. Estimates of loci under selection have generally been small in number (tens of loci; [89–91]) and largely circumscribed to immune-related genes [92]. However, recent studies using more powerful methods and larger sample sizes suggest that balancing selection, including fluctuating selection, directly maintains hundreds of independent causal loci [2,3,93–95], suggesting a more prominent role for balancing selection in maintaining functional genetic variation than has been previously appreciated.

To date, the strongest empirical evidence for multilocus fluctuating selection at the molecular level comes from studies of *D. melanogaster* populations from temperate North American and European environments. A study of North American *D. melanogaster* conducted by Bergland and colleagues identified approximately 1750 sites fluctuating seasonally from a total of ~500 000 surveyed SNPs (false discovery rate <0.3) in a Pennsylvanian population sampled in the spring and fall seasons across 3 years in succession [2] (Figure 1, Key figure). The frequency of selected loci changed by an average of 20% between spring and fall across the 3 years, equating to selection coefficients of 5–50% per locus per generation. When accounting for the limited statistical power of their study, the authors estimate that the total number of sites that cycle either as a direct result of seasonally fluctuating selection or through linkage to such a site could be ten times the number identified. Bergland and colleagues also found evidence that many seasonal SNPs were shared polymorphisms with *Drosophila simulans*, a sister species to *D. melanogaster* that diverged several million years ago [14], implying that these loci had either been segregating prior to their divergence or are recurrent mutations that are maintained by oscillating seasonal selection pressures over millions of years. Nonetheless, seasonal changes in life history traits measured for *D. simulans* and *D. melanogaster* populations sampled from the same Pennsylvanian orchard showed substantial interspecies differences, suggesting that these putative shared seasonal SNPs may not be maintained by selection pressures that are common to both species [15].

A subsequent study examined allele frequency fluctuations within 20 *D. melanogaster* populations distributed across North America and Europe [3], with Machado and colleagues reporting average shifts of 4–8% and associated selection coefficients of 10–30% per season, among the top 1% (i.e., most significant) of all common ~775 000 SNPs surveyed. Interestingly, the fluctuating SNPs identified by Bergland and colleagues in the Pennsylvanian population were only slightly enriched among the top 1% of SNPs identified in this study, and this enrichment was not statistically significant using a permutation approach ($p_{\text{perm}} = 0.0512$). This discrepancy may be due to the addition of many more populations in the more recent study, leading to increased statistical sensitivity for alleles exhibiting small but consistent seasonal fluctuations across

Glossary

Adaptive tracking: continuous adaptation in response to a rapid environmental change.
Allozyme: enzymes that have the same function but are structurally different.
Antagonistic pleiotropy: when loci contribute to multiple traits with contrasting effects.
Background selection: loss of neutral genetic variations due to negative selection of linked deleterious alleles.
Balancing selection: classically defined as the maintenance of genetic variants at intermediate frequencies over long periods of time. More broadly defined, it is an evolutionary force that leads fitness-affecting variants to segregate within a population longer than expected.
Boom–bust: exponential population growth (boom; summer season) followed by collapse (bust; winter season) where selection on the beneficial summer allele weakens at higher population densities.
Chromosomal inversion: a type of mutation where a segment of DNA has been flipped in place in the genome.
Diminishing-returns epistasis: a form of epistasis where, as more loci with beneficial alleles are present in a genome, there is less fitness advantage gained from each additional beneficial allele.
Dominance: describes the relationship between the phenotype and the genotype at a diploid locus in heterozygotes; quantified by the dominance coefficient d. In this context, $d > 0.5$ indicates that the allele is dominant and masks the alternative allele, whereas $d < 0.5$ indicates that the allele is recessive.
Dominance modifier: an allele, or epigenetic process, that changes the dominance of another locus.
Effective population size (N_e): the hypothetical number of individuals in an idealized Wright–Fisher population that has the same rate of genetic drift as the population of interest.
Fitness: a measure of reproductive success, or the likelihood that an individual or genotype can survive long enough to reproduce or be passed on to the next generation.
Fluctuating selection: when the strength and/or direction of selection shifts over time.
Frequency-dependent selection: where fitness depends on the frequency

Key figure

Detecting loci with seasonally fluctuating allele frequencies

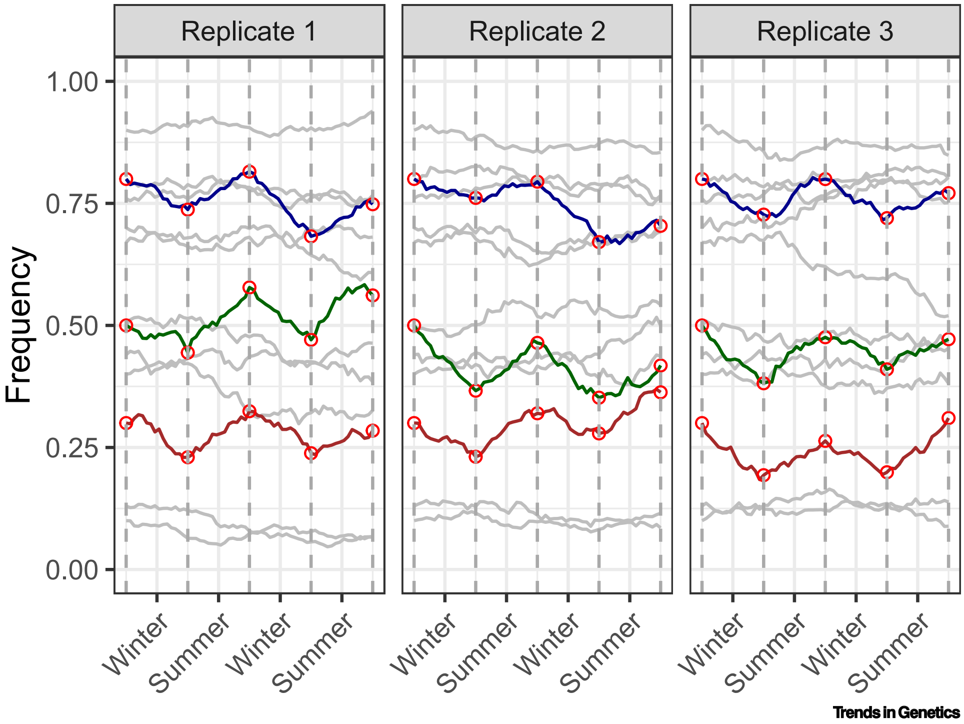


Figure 1. Allele frequency trajectories were simulated under seasonally oscillating selection pressures. Three loci displaying parallel seasonal allele frequency oscillations within replicates (i.e., across years) and between replicates are highlighted (red, green, and blue lines). This repeated seasonal change in frequency across replicates is the underlying pattern used to statistically identify seasonally fluctuating loci. The null hypothesis is that neutral loci unlinked to selected sites (grey trajectories) will not experience such regular seasonal oscillations. Statistical analyses have employed a binomial logit-linked generalized linear model (GLM) that regresses the frequency of each locus on season (i.e., spring and fall) encoded as a dummy variable [2,3,13]. More complex statistical models that account for repeated measures produce similar results [2,3]. The Bergland *et al.* study [2] compared parallel shifts measured across successive seasonal time points, whereas both the Machado *et al.* [3] and the Rudman *et al.* [13] studies had replication at the temporal and population levels. While genetic drift (e.g., grey lines) and migration from neighboring demes violate model assumptions, these were rejected as likely explanations for the observed seasonal frequency fluctuations based on simulations [2]. Statistical models that explicitly account for genetic drift and time-varying selection pressures are under active development and could be applied in the future to more robustly infer selected polymorphisms and quantify the contribution of fluctuating selection to allele frequency changes across the genome [28,108].

the majority of sampled populations (Figure 1), while being less likely to detect seasonal fluctuations that are population-specific[3]. Nonetheless, the study demonstrated that seasonal adaptation is a general phenomenon that impacts numerous loci in temperate fruit fly populations.

D. melanogaster harbors several large **chromosomal inversions** that have been shown to influence adaptive clinal variation across multiple continents [16], suggesting that these structural variants might also contribute to the quantity and dynamics of fluctuating loci reported for this species. However, while an excess of seasonally fluctuating SNPs was found in several different inversions by Machado and colleagues [3], the identity of enriched inversions tends to be

of the phenotype or genotype in the population.

Genetic drift: shifts in allele frequency in a population over time due to random sampling of alleles for the next generation from the gene pool of the current generation.

Geometric mean fitness: the geometric mean of the fitnesses of a genotype across generations. It is the relevant criterion for the maintenance of polymorphism in models with discrete generations.

Harmonic mean fitness: the reciprocal of the mean of the reciprocals of the fitnesses of a genotype across space; a key quantity in models of spatially maintained genetic variation.

Heterozygote advantage: also termed overdominance; when the heterozygote genotype has higher fitness than either homozygous genotype.

Hitchhiking: where the linked flanking regions of loci under selection are carried along with the selected allele to a high frequency.

Positive epistasis: a form of epistasis where fitness benefit per allele increases with each additional beneficial allele across loci.

Positive selection: selection on a genetic variant that confers a beneficial trait in a given environment, causing it to increase in frequency.

Protection from selection: also known as the storage effect; each season, a proportion of the population is replaced by juveniles. The remaining adult individuals are not subject to selection.

Sexually antagonistic selection: when a trait that is favorable in one sex is unfavorable in the other.

Tajima's D: a neutrality test statistic that measures the difference in mean number of pairwise nucleotide differences and the number of segregating sites across a region. Negative values signify an abundance of low-frequency variants, associated with positive selection or population expansion. An excess of intermediate-frequency alleles, associated with balancing selection, population bottlenecks, or structure, results in a positive value.

inconsistent across different studies [3,17,18], and no such enrichment was observed among segregating inversions in the North American *D. melanogaster* population studied by Bergland and colleagues [2]. This suggests that the role of inversions may be strongly dependent on the local population genetic profile.

Attempts to identify the potential environmental pressures underlying the seasonally fluctuating *Drosophila* loci also suggest that local factors may predominate over any shared ecological drivers [2,3,18,19]. For instance, allele frequency changes were well predicted by maximum temperature prior to sampling in a North American *D. melanogaster* population but not in European populations, where average temperature and humidity were better predictors [18]. Accordingly, adaptive seasonal allele fluctuations in *D. melanogaster* appear to be strongly influenced by local genetic and environmental factors that invoke highly population-specific dynamics, and this might partly explain the lack of common candidate seasonal SNPs observed across the different studies.

Experimental validation of rapid seasonal adaptation

Recently, a novel **adaptive tracking** field experiment involving outbred *D. melanogaster* populations was deployed to explicitly evaluate the role of selection in maintaining seasonally oscillating loci and its associated **hitchhiking** effects [13]. The experimental design involved rearing fruit flies in outdoor cages that contained features of a natural orchard – for example, fruit trees, natural ground cover, and insect and microbial community – along with regularly replenished food and egg-laying substrate. This aimed to allow the experiment to capture natural shifts as a result of local ecological features rather than experimental artefacts. Because the experiment was designed to preclude opportunities for gene flow events, all allele frequency changes could be attributed to either genetic drift or selection acting on pre-existing variants. Around 9000 SNPs exhibited significant parallel allele frequency shifts across ten replicated populations measured at multiple points between midsummer and late fall in a single year, with changes ranging from 2% to 8% between consecutive sampling periods and up to 5% when comparing the first and final samples taken during the experiment.

Applying a statistical approach that accounts for linkage among the putatively selected sites resulted in a total of 165 unlinked independent genomic clusters that exhibited parallel directional changes indicative of rapid adaptation to seasonal changes in selection pressures over the year, a pattern consistent with strong fluctuating selection. Remarkably, selection at these loci was inferred to have impacted the frequencies at >60% of the 1.9 million genome-wide SNPs screened during the study. Only three clusters were found to be strongly linked with known inversions, indicating that the seasonal signal seen in this study is not strongly driven by common segregating inversions. Consistent with theoretical results [20,21], simulations under a truncation selection model showed that the observed frequency shifts are feasible despite the large number of independently selected loci (i.e., 4.5 loci per chromosome per month, changing at least 2% in frequency) that are competing against each other [13]. Combined with the two studies of wild *Drosophila* populations, these results provide strong evidence for fluctuating selection targeting at least hundreds of independent loci in *Drosophila* populations in temperate environments. They indicate that fluctuating selection is likely to affect allele frequency changes at linked neutral alleles across a large portion of the genome, and suggest that it also has a major role in shaping genome-wide diversity in these populations. Interestingly, while this study provides further support for multilocus fluctuating selection being a common feature in *Drosophila* populations living in temperate climates, we found no significant overlap between the candidate SNPs reported in Bergland *et al.* [2] and Machado *et al.* [3] and the genomic clusters of Rudman *et al.* [13] (Bergland–Rudman overlap $p_{\text{perm}} = 0.813$, Machado–Rudman overlap $p_{\text{perm}} = 0.445$). While this lack of overlap suggests that a substantial fraction of the seasonally adapting loci might be population specific and/or temporally constrained (see Outstanding questions), it remains unknown to what extent this lack of replicability was caused by the imperfect mirroring of selection pressures between the experimental regime and natural environments.

Evidence for fluctuating selection beyond *Drosophila*

While most evidence for multilocus fluctuating selection comes from observations of *Drosophila* populations, fluctuating selection has been identified in several other species, suggesting it may be found throughout all life.

A study of non-biting midge (*Chironomus riparius*) over a cold-snap period found evidence of fluctuating selection at 19 SNPs, of which ten were unlinked [12]. Frequency changes of at least 50% were observed for all ten independent SNPs, with all but one returning to their pre-cold-snap frequency after 6 months. **Balancing selection** was inferred at four of the SNPs using **Tajima's D**, with one other SNP showing signs of a recent selective sweep. A separate study of the same species sampled seasonally over 3 years also found that nearly 360 000 SNPs were impacted by selection (among ~22.7 million SNPs), with alleles changing frequency by at least 15% between two consecutive sampling periods or the first and last sampling points. Notably, while some loci were found to switch direction in concert with environmental changes, the majority did not covary with seasonal change [11] (Box 2). Rapid adaptation was also observed in a study of six populations of threespine stickleback (*Gasterosteus aculeatus*) inhabiting bar-built estuaries that undergo seasonal environmental changes due to intermittent connectivity to the ocean. Analyses of pooled sequencing data sampled in spring and fall of 2016 revealed significant parallel allele frequency shifts at thousands of loci across the six populations [22]. Functional enrichment analysis of the candidate genes suggests rapid osmoregulatory adaptation to temporal changes in salinity. Similar patterns were reported for coastal *Arabidopsis thaliana* populations, where alleles conferring differential levels of salinity tolerance were found to be maintained by annual fluctuations in soil salinity levels [23].

Because of a lack of long-term genetic time-series data for most species, direct molecular evidence for fluctuating selection pressures operating over super-seasonal ecological timescales is largely limited to species for which ancient DNA is available. For instance, alleles associated with leopard complex spotting (LP), a speckled coat pattern, were found to fluctuate in frequency in ancient horse genomes between the Late Pleistocene (approximately 17 000 years ago) and the Iron Age [24]. This is suspected to result from waves of artificial selection for the speckled coat pattern favoring the LP allele, which eventually becomes detrimental as LP homozygotes exhibit congenital night blindness. In humans, studies of ancient genomes have revealed that selection pressures often vary over millennial timescales, with some introgressed Neanderthal alleles persisting at low frequencies for tens of thousands of years before being targeted by selection

Box 2. Non-oscillating or aperiodic fluctuating selection

Natural environmental fluctuations can follow periodic cycles (diurnal, seasonal, or pluriannual) but also exhibit random variation or noise that is aperiodic. While such non-oscillatory fluctuations do not exhibit regular temporal changes by nature, they can result in serial changes in the direction of selection (i.e., where the beneficial allele alternates through time) that are sufficiently frequent to preserve genetic variation at the selected locus [8,11]. Accordingly, non-oscillating environmental fluctuations are unlikely to result in the distinct frequency fluctuations observed in *Drosophila* but might still affect genetic diversity and be detected in genetic time-series data. For example, time-series data for the non-biting midge (*Chironomus riparius*) shows non-neutral changes in allele frequency at hundreds of loci across the genome, which cluster in distinct temporal patterns. In this case, seasonal patterns were of relatively minor importance, and only a few temporal patterns could be related to measured environmental variables [11]. Although our review largely focuses on cyclical environmental change, theoretical work on randomly changing adaptive environments has demonstrated that non-oscillatory fluctuations in selection pressures can also maintain genetic polymorphism and phenotypic/genetic variance of quantitative traits under certain conditions [38,85,96].

[25,26]. Similarly, alleles associated with adaptation to agricultural diets were initially introduced into early European farming populations at the start of the Neolithic, but only became strongly selected some 5000 years later in the Bronze Age [27,28]. Furthermore, recent research has demonstrated that causative variants for many human traits and diseases have population-specific effects [29,30], implying the intensity of the underlying selection pressures has changed over time in at least some of the studied populations [31].

Beyond genomic data, evidence for temporally varying selection pressures operating at the phenotypic level has been reported in studies of vertebrate species that measured trait and **fitness** components over time [8]. For example, the strength of selection on breeding dates was estimated for 31 populations of 21 species of birds and mammals, showing that variation in the strength of selection and/or fluctuations of an optimum phenotype had strong statistical support across all taxa [32].

These examples suggest that fluctuations in the strength and direction of selection may be a reasonably common feature for a wide variety of species and that these fluctuations can be tracked through time and are influencing the allele frequency dynamics of many genomic loci. However, in many of these cases, the lack of long-term temporal coverage means that it remains unclear whether the fluctuating selection pressures also alter the direction of selection frequently enough to avoid the fixation of adaptive alleles – a necessary condition to maintain polymorphisms in models of fluctuating selection [33]. In the future, the analysis of ancient DNA datasets covering successive ecological and/or climate cycles and long-term field studies will help improve our understanding of the environmental drivers of fluctuating selection pressures and their temporal characteristics.

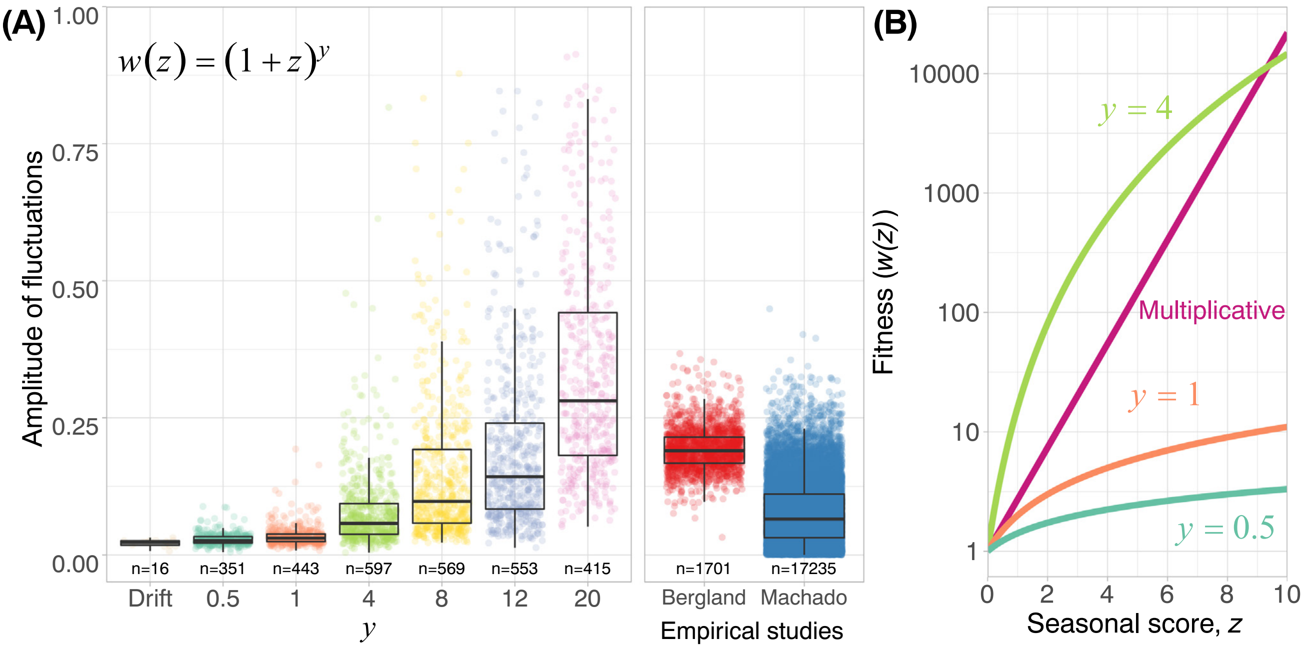
Theoretical models of fluctuating selection

Theoretical studies have aimed to evaluate the conditions under which genetic variants are maintained in fluctuating environments. Haldane and Jayakar first proposed a general condition for a temporally fluctuating polymorphism in a biallelic single-locus model [33] where the favored allele alternates across two opposing environments (e.g., hot and cold). Stable polymorphism at a single locus requires the heterozygote to have a greater **geometric mean fitness** than the homozygous alternatives [33]. This contrasts with spatial selection models, where the difference in **harmonic mean fitness** between genotypes is key [34–36]. Since this seminal study by Haldane and Jayakar, fluctuating selection has been explored through models of temporally fluctuating selection coefficients [37] as well as Gillespie's SAS-CFF (stochastic additive scale–concave fitness function) model, which was designed to provide a mechanistic explanation for genetic variation in enzymes [38–48]. The SAS-CFF model uses a SAS to imitate enzyme activity under a random continuous environment, with the enzyme activity being mapped to a CFF. These properties result in random fluctuations in selection pressures (Box 2), which produce stable polymorphic loci when the average effect of each allele is the same and only weakly correlated across different environments [38].

Although a single stable polymorphism can be well explained by these models, extending them to the multilocus case (i.e., potentially explaining the hundreds of fluctuating loci seen in *Drosophila*) is nontrivial [4]. For instance, the evolutionary applicability of genetic models that permit non-additive loci and multiplicative epistasis was questioned for requiring seemingly unrealistic levels of genetic load (i.e., whereby some individuals would have to produce an astronomically large number of offspring to avoid population extinction) [49–51]. By contrast, models of fluctuating selection where loci contribute additively to a trait can maintain polymorphism at only one or two loci [4,52]. Accordingly, multilocus fluctuating selection has long been considered unrealistic as a mechanism for the maintenance of a large number of polymorphic sites [4].

In light of the compelling empirical evidence emerging from population genomic studies of *Drosophila* [2], recent theoretical work has re-evaluated the plausibility of multilocus fluctuating selection by drawing on a wider class of selection models that include various forms of **dominance** and epistasis [4]. In particular, the 'segregation lift' (SL) model proposed by Wittmann and colleagues can maintain polymorphism at hundreds of loci under seasonally oscillating selection pressures without generating unrealistic levels of genetic load (Figure 2) [4]. The SL model decomposes fitness into two parts: (i) the seasonal score (z) contributed by each locus, scaled by a seasonal dominance coefficient; and (ii) a fitness function [$w(z)$] that allows for epistasis across selected loci and is constant across seasons (Figure 2). Both **positive** and **diminishing-returns epistasis** were examined; however, maintenance of long-term fluctuating loci under positive epistasis required that seasonally favored alleles are almost completely dominant (i.e., extreme dominance reversal, Box 3; dominance approaches 1 as the number of loci increases), resulting in large changes in dominance between seasons. By contrast, with diminishing-returns epistasis, more moderate changes in dominance were permissible – as the number of loci increases, permissible dominance values approach 0.5 – making diminishing-returns epistasis more plausible overall [4]. Moreover, beneficial mutations often exhibit diminishing-returns epistasis in empirical studies [53–55].

Wittmann and colleagues also investigated a more complex version of their SL model that allowed dominance and effect sizes of selected loci to take random values that are potentially asymmetric



Trends in Genetics

Figure 2. Amplitudes of allele fluctuations from empirical and simulated data. (A) Boxplot of amplitudes of allele frequency fluctuations of unlinked seasonally selected loci under Wittmann and colleagues' complex segregation lift (SL) model [4] with varying epistasis values (y parameter) in a population of 10 000 individuals with ten generations in each season. A total of 1000 loci (ten replicates of 100 loci) were simulated for each y value, with the number of fluctuating loci (i.e., loci still segregating after 90 000 generations) shown beneath each box. All loci have a combined summer and winter dominance greater than 0.5, the critical dominance value for stable fluctuations, which is used to generate the seasonal score [$z = \sum_{l=1}^{L} c_l$, where c_l is the contribution of each locus (i.e., the product of the effect size and the dominance of the locus)]. By varying y, the mean amplitude of the fluctuations can encompass the range of mean amplitudes reported in natural *Drosophila melanogaster* populations by both Bergland *et al.* [2] and Machado *et al.* [3], the distributions of which are shown. X-chromosome SNPs are excluded from the Bergland *et al.* distribution and were not analyzed in the Machado study. (B) Note that, as y increases, epistasis becomes more negative while the strength of per-locus selection increases. This is because log[$w(z)$] is the relevant scale to evaluate epistasis [4].

Box 3. Dominance reversal

The concept of dominance reversal was originally proposed by Sewall Wright in 1915 [97] and has since been discussed primarily in terms of its role in **antagonistic pleiotropy** and sexually antagonistic selection [10,98–104]. Loci characterized under both criteria exhibit fitness trade-offs whereby the fitness effect of an allele switches from positive to negative across environments (with the different sexes being the relevant 'environment' for the latter criterion; Figure I) [98,105]. Dominance reversals can maintain polymorphism at loci exhibiting fitness trade-offs by enabling a net heterozygote advantage; that is, the identity of the beneficial allele alternates across environments but dominance always favors the beneficial allele, sufficiently masking the effects of the deleterious allele in heterozygotes to promote polymorphism [101].

The prevalence of dominance reversal in natural systems remains largely unknown, though it has been observed for polymorphic loci related to salinity tolerance in the copepod *Eurytemora affinis*, with these loci carrying alleles that are simultaneously beneficial and completely dominant in either saltwater or freshwater environments [106]. Dominance reversals have also been observed to impact the expression levels of nearly 1400 different genes in experimental *Drosophila melanogaster* populations evolving under two different temperature regimes, likely as a result of temperature-mediated stress affecting *cis* and *trans* regulation [107]. Around two-thirds of the genes experiencing dominance reversals showed evidence of *trans*-regulatory control, with two of the 13 identified transcription factors also experiencing temperature-dependent dominance reversals themselves [107]. Regulatory mechanisms have also been explored as potential **dominance modifiers** in dominance reversals associated with sexually antagonistic selection, with simulations showing that the regulatory properties of the dominance modifier (in this case, a *cis*-regulatory binding site) induced reverse dominance in allele expression between the sexes [105]. This indicates that dominance reversals are a plausible mechanism in natural populations, although its role in maintaining variation in the presence of fluctuating selection remains to be empirically verified.

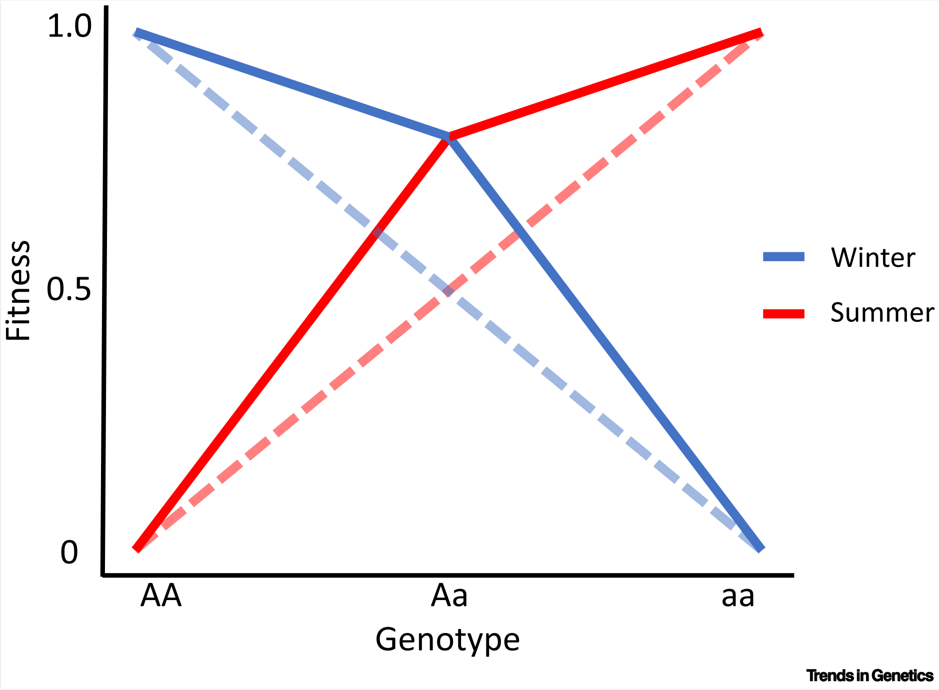
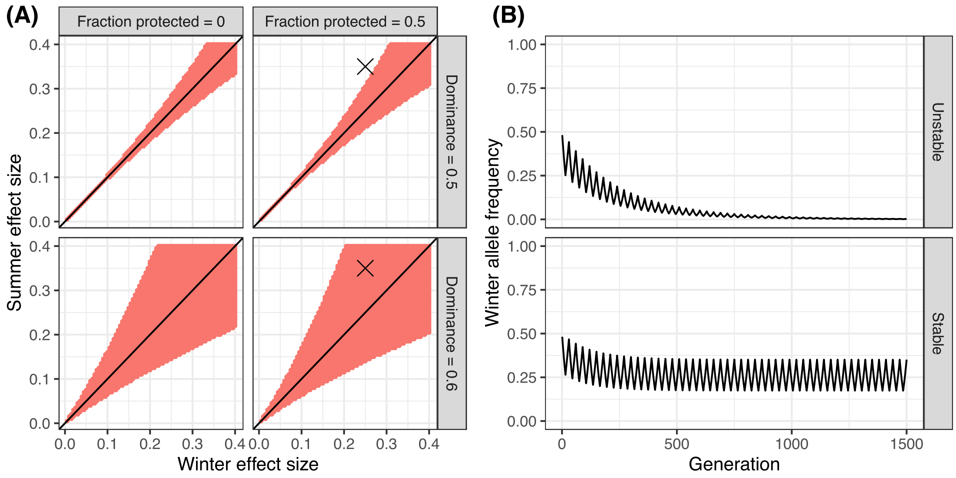


Figure I. Beneficial reversal of dominance in a two-season environment (unbroken lines). The A allele is dominant and beneficial in the winter and becomes recessive and deleterious in the summer. Fitness for the additive case is shown with broken lines. The figure shows symmetrical patterns of dominance across seasons, but it is possible for dominance to be asymmetrical (although the geometric mean fitness across generations of the heterozygote must be larger than that of either homozygote to sustain a polymorphic locus).

across seasons [4]. In this generalized setting, the authors found that stable polymorphisms were possible as long as the arithmetic mean dominance between seasons was greater than 0.5 (i.e., the seasonally favored allele is on average dominant across the two seasons). Accordingly, the SL model suggests that fluctuating selection can maintain hundreds of polymorphic loci under quite general conditions. Notably, less than 10% of simulated loci were considered detectable (i.e., had shifted 5% in the expected direction in at least half of the evaluated seasons), with these loci typically having the greatest effect size. Even so, by merely varying the epistasis parameter, loci under this model produce allele frequency fluctuations comparable with empirical observations in natural *Drosophila* populations (Figure 2).

Ecological mechanisms for fluctuating selection

Reversal of dominance (Box 3) is fundamental to the SL model: it allows the maintenance of genetic variation without having to assume unrealistically strong selection pressures [6]. Bertram and Masel [6] examined whether ecological mechanisms can also produce balanced polymorphisms in fluctuating environments without having to assume dominance reversal. In conjunction with the genetic mechanism of dominance reversal, they investigated two ecological mechanisms – **boom–bust** demographies and **protection from selection** – both of which are known to lead to low-frequency alleles being favored over high-frequency alleles, a requirement of stable polymorphisms [6]. When modeling the dynamics at a single locus with alleles alternately favored in a binary seasonal environment (i.e., summer and winter), both ecological and genetic mechanisms were capable of stabilizing alleles with strong fitness effects, provided that the effect sizes are relatively similar across seasons (Figure 3). However, only dominance reversal was able to maintain alleles with weak fitness effects. Nonetheless, the authors conclude that both genetic and ecological mechanisms, possibly in combination, may plausibly maintain individual loci exhibiting allele frequency changes consistent with empirical observations in *D. melanogaster* (i.e., where selection pressures are strong). Further, the



(C)	Summer homozygote	Heterozygote	Winter homozygote
Summer	$\omega_{SS}(S) = 1 + a_S$	$\omega_{SW}(S) = 1 + da_S$	$\omega_{WW}(S) = 1$
Winter	$\omega_{SS}(W) = 1$	$\omega_{SW}(W) = 1 + da_W$	$\omega_{WW}(W) = 1 + a_W$

Trends in Genetics

Figure 3. Protection from selection model. (A) Effect sizes for summer and winter alleles that result in stably fluctuating loci (red region in each subpanel) under different fractions of protection (0, 0.5) and additive ($d = 0.5$) or reversal of dominance ($d = 0.6$) models. (B) Allele trajectories when 50% of the population is protected from selection. The black cross in (A) shows the seasonal effect sizes (i.e., both panels: winter = 2.3, summer = 3.5) and dominance values (i.e., top panel = 0.5, bottom panel = 0.6) for the adjacent allele trajectories plotted in (B). (C) Fitness equations used in (A,B), where $a_{s/w}$ represents the fitness benefit of the seasonal allele and d signifies the dominance of the seasonal allele [5,6].

authors suggest that ecological mechanisms widen the scope for temporal variability to balance polymorphism, although the study did not explicitly model multilocus scenarios [6].

The 'protection from selection' mechanism has been extended to the multilocus case in a different study [7] that includes a 'refuge' subpopulation that is protected from cyclically fluctuating selection pressures. This model was shown to maintain a moderate number of selected polymorphisms, but in its current formulation is not able to explain the large number of fluctuating loci observed in North American *Drosophila* [2]. However, it remains to be investigated whether model modifications could promote the maintenance of larger numbers of segregating loci [7], which would demonstrate that dominance reversal is not essential for sustaining hundreds of fluctuating alleles.

Fluctuating selection affects genome-wide diversity

In addition to directly impacting the evolution of selected loci, fluctuating selection may exert considerable influence over surrounding neutral genetic variation. A longstanding debate amongst population geneticists concerns whether the cumulative effects of selection have a major role in determining genome-wide levels of diversity observed within and among different species [56,57]. The genetic hallmarks of **positive** and **background selection** on flanking regions have been well investigated; both selection modes generally reduce levels of linked genetic diversity with the most pronounced reductions occurring in regions with low recombination rates [58,59]. By contrast, classical models of balancing selection lead to an increase in diversity at tightly linked regions but have no effect on diversity in more distant regions [60].

The effect of fluctuating selection on genetic diversity at linked sites is much less well investigated (but see [61–63]). In terms of diversity patterns, fluctuating selection produces signatures consistent with both balancing selection and selective sweeps (i.e., strong positive selection on a single beneficial locus): it increases diversity close to the selected site but decreases diversity further away [5,7,63]. Intriguingly, a fluctuating locus can also diminish genetic variation at unlinked regions (e.g., different chromosomes [5,64]), a consequence of the strong recurrent bottlenecks created by the skewed fitness distribution across individuals after environmental change (whereby the majority of offspring are produced by a small number of individuals [61]). A recent theoretical study suggests that this genome-wide diversity-reducing effect outweighs the increase in diversity in regions tightly linked to the selected sites, predicting a substantial reduction in the diversity in species experiencing strong multilocus fluctuating selection [5]. The scale of this reduction increases with the magnitude of allele frequency fluctuations at the selected loci [5,63], although even small fluctuations can cause large genomic reductions in neutral diversity when their effects aggregate across many loci [5,61].

Importantly, subtle allele frequency fluctuations can be difficult to detect in population genetic data using standard approaches and thus might often be missed or misinterpreted as classical balancing selection. Accordingly, large-sample-size time-series data will be necessary to quantify the abundance and magnitude of fluctuating selection across the genome in various species and to provide insights on the fundamental parameters needed to model its effect on linked neutral diversity. Empirical support for the theoretical predictions comes from an evolve-and-resequence study of *D. melanogaster* populations adapting to either constant or spatially/temporally fluctuating salt and cadmium environments [65]. Among the different regimes, the lowest levels of neutral diversity were found in replicated populations exposed to temporally fluctuating environments, suggesting that the fluctuating regime indeed reduces genome-wide diversity.

In sum, a handful of theoretical and experimental results indicate that fluctuating selection could be a major but currently underappreciated factor shaping levels of genetic diversity in natural populations. If so, this would have important implications for the resolution of Lewontin's paradox (i.e., the observation that levels of diversity across metazoans vary by only two orders of magnitude while census population sizes vary over several [57,66]). For instance, when exposed to periodically changing environments, species with a large census population size might experience sequential genetic bottlenecks caused by rapid adaptation to changing environments leading to a small local **effective population size (N_e)** (as shown in [18,67] for *Drosophila*), whereas species defined by smaller populations and longer generation times might maintain higher levels of genetic diversity by virtue of being less able to adapt to short-term environmental fluctuations. Therefore, if fluctuating selection is widespread among species, this mechanism could partially resolve Lewontin's paradox by reducing neutral genetic diversity in species in relation to their census population size and generation time [57]. Similarly, fluctuating selection might also contribute to genome-wide levels of linkage disequilibrium (LD) in various species. Indeed, it was suggested that recurrent selection at multiple loci may help to explain the observed excess in long-range LD in natural *D. melanogaster* populations beyond expectations under purely demographic models [68,69].

Concluding remarks and future perspectives

Selection in natural populations is likely to be a dynamic interplay between spatially and temporally varying selection pressures that shape the distribution of functional variation. However, temporally fluctuating selection remains understudied relative to adaptation to spatially heterogeneous environments. Recent results from cosmopolitan *D. melanogaster* populations reporting hundreds to thousands of seasonally selected loci have helped to revive interest in the role of temporally fluctuating selection in adaptation and the maintenance of genetic variation [2,3,13]. Although the concept of fluctuating selection has been around for more than 100 years, only in the past decade have advances in whole-genome sequencing allowed the detection of causal loci and the quantification of their dynamics from genetic time-series data. It is possible that many balanced polymorphisms previously identified in population genetic studies are actually the targets of fluctuating selection rather than examples of classical balanced loci (i.e., where two alleles are maintained at constant frequencies); the similarity of the surrounding genetic footprints left by the two modes of selection is expected to make the two forms difficult to distinguish [5]. Together with evidence of fluctuating selection in a range of species, this suggests that multilocus fluctuating selection may be far more common than previously thought [70]. Accordingly, the development of novel statistical methods to discriminate balanced and fluctuating loci in population genetic studies and the generation of suitable time series datasets for diverse taxa are sorely needed to establish the prevalence and impact of fluctuating selection across the tree of life.

There are still many theoretical and empirical aspects of fluctuating selection and oscillating loci of which we remain largely ignorant (see Outstanding questions). For example, while a significant fraction of fluctuating polymorphisms observed in *D. melanogaster* is shared with sister species *D. simulans* [2], it is plausible that the bulk of these variants are only transient and subject to frequent turnover. This may also explain the lack of overlap in candidate loci between studies of cosmopolitan *D. melanogaster* populations [2,3,13]. Further, the influence of fluctuating selection on genome-wide diversity remains relatively unknown, as previous theoretical and empirical investigations have predominantly focused on the significance of selective sweeps and background selection [59,71]. The recent availability of fast and powerful population genomic simulators [72,73] provides a promising avenue to compare models of fluctuating selection with empirical observations and gain a better understanding of the dynamics at play. In addition, ecological mechanisms remain understudied in the light of recent findings showing that they are a plausible basis for fluctuating loci [6,7], and further investigation is needed to elucidate their role relative to genetic mechanisms like dominance reversal [6].

Outstanding questions

How long are alleles under fluctuating selection typically maintained in natural populations?

How do fluctuating alleles become established? Is it through *de novo* mutation or through introgression from populations in extreme environments?

How prevalent is multilocus fluctuating selection in species other than *Drosophila* and what are the main environmental drivers?

Is there power to discriminate the population genetic signatures of fluctuating selection from those of simple forms of balancing selection or soft selective sweeps?

Is reversal of dominance the main mechanism maintaining alleles under fluctuating selection or do other mechanisms also play a role?

What is the relevance of genes under fluctuating selection for adaptation to massive environmental shifts such as anthropogenic climate change?

To what degree are fluctuating alleles and their environmental pressures shared between species?

Finally, fluctuating selection is potentially an important contributor to genetic variance in fitness. A recent meta-analysis based on an improved statistical approach has proposed that a substantial amount of additive genetic variance in fitness in wild bird and mammal populations had been missed by previous studies, such that additive genetic variance is much larger than previously thought [74]. A substantial fraction of this variance is likely to be maintained by selection [75], with fluctuating selection being a plausible candidate. Ultimately, fluctuating selection might be a major driver of genetic diversity and an important mechanism enabling rapid adaptation to changing climatic conditions and, if so, elucidation of its role will become increasingly relevant for future conservation efforts to protect endangered species [76,77].

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Declaration of interests

No interests are declared by the authors.

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