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Perspective

PERSPECTIVE

What have we learned about sleep from selective breeding strategies?

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Abstract

Selective breeding is a classic technique that enables an experimenter to modify a heritable target trait as desired. Direct selective breeding for extreme sleep and circadian phenotypes in flies successfully alters these behaviors, and sleep and circadian perturbations emerge as correlated responses to selection for other traits in mice, rats, and dogs. The application of sequencing technologies to the process of selective breeding identifies the genetic network impacting the selected trait in a holistic way. Breeding techniques preserve the extreme phenotypes generated during selective breeding, generating community resources for further functional testing. Selective breeding is thus a unique strategy that can explore the phenotypic limits of sleep and circadian behavior, discover correlated responses of traits having shared genetic architecture with the target trait, identify naturally-occurring genomic variants and gene expression changes that affect trait variability, and pinpoint genes with conserved roles.

Statement of Significance

Selective breeding makes the improvement of animals and crops possible. Recent work couples this classic breeding strategy with sequencing technology to trace the genomic and gene expression changes underlying observed changes in sleep and circadian behavior. Selective breeding emerges as a comprehensive approach for the identification of genetic networks and the detection of conserved genomic variants for sleep.

Key words: selective breeding; artificial selection; sleep; circadian rhythms; Drosophila; mice; rats; dogs

Introduction

For thousands of years human beings have used selective breeding to improve the characteristics of domesticated animals and crops [1]. Selective breeding has a prominent role in the history of experimental biology and genetics: the results of selective breeding experiments underlie Darwin's theory of natural selection [2]. Selective breeding involves the active participation of a breeder or experimenter with the goal of altering a specific phenotype. To alter a target trait, an experimenter uses a three-step process. First, the experimenter measures the target trait in an outbred population. Second, the experimenter chooses males and females with the most extreme (highest or lowest) values of the target trait as the parents for the next generation and allows them to mate. Third, the experimenter measures the target trait in the resulting

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progeny to assess the response to selection. Selection continues in this fashion each generation. Usually the experimenter chooses a consistent proportion of the individuals measured each generation as the breeding parents, referred to as the intensity of selection [1]. Selective breeding differs from evolved changes that occur when populations are exposed to different environments in nature, such as the eye loss, reduced pigmentation, and reduced sleep observed in the cave-dwelling Mexican tetra (*Astyanax mexicanus*) compared to surface populations [3]. Selective breeding is also distinct from laboratory evolution, where an experimenter exposes the population to certain conditions (i.e. high temperatures) but does not choose which animals will breed in successive generations [4]. Instead, selective breeding relies on the intent of an experimenter to "push" the values of a trait to extremes not normally observed in nature by choosing a small percentage of animals at either end of the phenotypic distribution for breeding.

Virtually any genetically variable trait is amenable to selective breeding, and in fact traits that respond to selective breeding confirm the presence of an underlying genetic basis [1]. The majority of sleep parameters studied to date, such as sleep duration, numbers of naps, average nap length, sleep latency, and day-to-day variability in sleep [5–17] are heritable, as are behaviors related to the circadian regulation of sleep such as circadian period, chronotype, and rhythmicity index [8, 18–21]. Furthermore, sleep characteristics related to human sleep disorders, such as insomnia symptoms, excessive daytime sleepiness, daytime napping, and sleep-disordered breathing [22–26] all have a heritable basis. Consequently, these heritable phenotypes should respond to selective breeding to the extent that they can be modeled in nonhuman animals.

Modern selective breeding experiments have a distinct advantage over earlier experiments. Using next-generation sequencing, it is now possible to trace the allele frequency changes (i.e. the change in the proportion of a given allelic variant in the population) and gene expression changes that respond to selective breeding. Doing so has led to new insights into the origins of genetic variation in sleep and will ultimately lead to an understanding of its function. In this perspective, I discuss what selective breeding experiments have discovered about sleep and circadian behaviors including some of the advantages and challenges, and outline a strategy for the future application of this technique.

Selective Breeding to Discover the Limits of What is Possible in Nature

Selective breeding enables the experimenter to explore the phenotypic limits of the target trait. Like experimental mutagenesis and CRISPR strategies, selective breeding modifies the underlying genome. However, selective breeding alters the allele frequencies of naturally-occurring polymorphic variants in the population rather than engineering synthetic alleles with altered function [1]. In this way, selective breeding capitalizes on the genetic variation already present in a population [1], providing a more realistic model of phenotypic potential.

Sleep and circadian parameters respond rapidly and dynamically to selective breeding (Table 1). Selection for 30 generations on a combination of increased sleep latency, reduced sleep bout duration, and increased activity produced flies with insomnia-like behavior as well as extremely short average sleep duration—less than 100 min in a 24-hour day [27], well below the average 923 min seen in natural populations [5]. Likewise, selection for long and short night sleep duration for 13 generations in flies produced divergent populations with a 9.97-hour difference in this parameter (Figure 1A) [28]. The extreme reduction in sleep duration achieved by these two studies was similar to that of engineered mutations in single genes on sleep duration [29–33], demonstrating that the combined effects of naturally occurring genomic variants on sleep can be large. Selective breeding modified traits under circadian regulation as well. Selective breeding changed the timing of adult fly eclosion (i.e. the emergence of the adult fly from the pupal stage) to specific early and late hourly windows during the day [34]. Furthermore, selective breeding produced robust nocturnal and diurnal activity patterns in flies [35]. One intriguing application of selective breeding is to produce animals sensitive or resistant to the effects of certain drugs, enabling a detailed understanding of their efficacy. For example, selective breeding based on the response of rats to γ-hydroxybutyric acid (GHB) created an animal model sensitive to the sedating effects of the drug [36]. One oft-noted challenge of selective breeding is the many generations required to produce populations with extreme phenotypes, making it a more favored strategy for species with short generation times. This need not be an intractable limitation, however, in some instances, the selective breeding has already been accomplished; the key is to recognize the utility of the resulting population in the study of sleep. For example, brachycephalic dog breeds—dogs selectively bred for a shortened muzzle and flattened face—have increased sleep disturbances and decreased sleep latency compared to other dog breeds, making them a readily available model for sleep-disordered breathing [37]. Selective breeding is thus a powerful strategy to effect change in sleep and circadian-related behaviors, demonstrating the limits of what is possible in nature.

Selective Breeding Reveals the Genetic Basis of Sleep and Circadian Behavior

It has recently been noted that the term sleep actually refers to a set of complex phenotypes, each with a polygenic basis [17, 42]. Consequently, genome-wide association studies and systems genetics approaches have detected hundreds of genes affecting different aspects of sleep and circadian behavior [5–7, 9–17, 19–26]. Furthermore, mutational screens in mice and flies revealed that a striking 14%–16% of mutations tested have quantitative effects on some aspect of sleep [42, 43] and 0.1%–0.2% of those tested have Mendelian (2–3 standard deviations) effects on sleep [29, 44]. As sleep is polygenic, it is likely that a single mutation does not act in isolation. At least two mutagenesis studies detected background modifiers to sleep-altering mutations in a single gene, for example [29, 45]. Adding to this complexity is the effect of circadian rhythms, which are thought to regulate sleep [46]. While the canonical molecular circadian clock genes are known, modifiers across the genome can alter circadian behavior [19–21, 47–49]. Selective breeding coupled with the measure of underlying molecular endophenotypes offers a solution to this challenge as outlined below.

As stated previously, selective breeding alters the allele frequencies of naturally occurring polymorphic variants within a population [1]. Accordingly, following the trajectories of allele frequency change per generation during the selection process via whole-genome sequencing facilitates the discovery of genomic variants that affect the target trait. This was done during