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Natural Variation of the Circadian Clock in *Neurospora*

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Abstract

Most living organisms on earth experience daily and expected changes from the rotation of the earth. For an organism, the ability to predict and prepare for incoming stresses or resources is a very important skill for survival. This cellular process of measuring daily time of the day is collectively called the circadian clock. Because of its fundamental role in survival in nature, there is a great interest in studying the natural variation of the circadian clock. However, characterizing the genetic and molecular mechanisms underlying natural variation of circadian clocks remains a challenging task. In this chapter, we will summarize the progress in studying natural variation of the circadian clock in the successful eukaryotic model *Neurospora*, which led to discovering many design principles of the molecular mechanisms of the eukaryotic circadian clock. Despite the success of the system in revealing the molecular mechanisms of the circadian clock, *Neurospora* has not been utilized to extensively study natural variation. We will review the challenges that hindered the natural variation studies in *Neurospora*, and how they were overcome. We will also review the advantages of *Neurospora* for natural variation studies. Since *Neurospora* is the model fungal species for circadian study, it represents over 5 million species of fungi on earth. These fungi play important roles in ecosystems on earth, and as such *Neurospora* could serve as an important model for understanding the ecological role of natural variation in fungal circadian clocks.

Keywords

Neurospora; circadian clocks; natural variation; ecotypes; adaptation

1. INTRODUCTION

Natural variation shapes the diversity of life. In nature, variation is selected by ambient environments, such as altitude, depth, seasons, latitude, and longitude, based on their impacts on fitness (Gaston, 2000). Thus, understanding the underlying principles of these variations and their fitness effect is one of the fundamental quests in biology. One main characteristic of all ambient environments to which most living organisms have adapted is daily cycles. Hot daytime follows cold nighttime, and bright daytime follows dark nighttime. Predicting the incoming environmental change is a crucial trait for an organism to escape or

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prepare for incoming danger, or to take full advantage of a favorable condition. Anticipating to the environmental change is only possible if one has a cellular device capable of measuring time: a biological clock. When the cellular clock runs with a period of about one day, it is called a circadian clock. Scientists tried to understand clock variation under controlled conditions using different circadian models other than *Neurospora*. In the 1980's, Pittendrigh and Takamura showed how latitudinal changes impact circadian parameters (phase, period and amplitude) using *Drosophila* (Pittendrigh, Kyner, & Takamura, 1991; Pittendrigh & Takamura, 1989). Later, several studies showed that naturally occurring polymorphs in the *Drosophila* period gene (*dper*) impact phenotypic variation in locomotion and temperature compensation (Low, Chen, Yildirim, & Edery, 2012; Low, Lim, Ko, & Edery, 2008; Rosato & Kyriacou, 2011; Sawyer et al., 1997). Additionally, the geographical variation of eclosion rhythms and adult diapause was reported (Lankinen & Forsman, 2006). A recent study has also reported latitude specific sleep variation in the *Drosophila* natural population (Svetec, Zhao, Saelao, Chiu, & Begun, 2015). Latitude-specific circadian variation has also been observed in other eukaryotes like plants, birds, and mammals. In *Arabidopsis*, natural allelic variations are associated with several circadian phenotypes like hypocotyl growth (Coluccio, Sanchez, Kasulin, Yanovsky, & Botto, 2011; Raschke et al., 2015), flowering time (Lempe et al., 2005; Press, Lanctot, & Queitsch, 2016), periodic leaf movements (Michael et al., 2003; Swarup et al., 1999) and temperature compensation (Edwards, Lynn, Gyula, Nagy, & Millar, 2005). These same allelic variations are also associated with the phenotypes for breeding time (Hau, 2001) and diurnal and seasonal variation of hormone secretion (Dawson, King, Bentley, & Ball, 2001; Reierth, Van't Hof, & Stokkan, 1999) in birds, and seasonal variation of melatonin circadian rhythms (Adamsson, Laike, & Morita, 2016) and latitudinal and seasonal specific qualitative and quantitative variations of sleep (Friborg, Bjorvatn, Amponsah, & Pallesen, 2012) in humans. Based on the population genetic studies, it has also been reported that human clock gene variants are associated with time of day preferences (Allebrandt & Roenneberg, 2008). Genome analysis of human populations at different latitudes revealed a correlation between different day-length (photoperiod) variations of clock genes and certain psychiatric disorders (Forni et al., 2014). Thus, research into natural variation of circadian clocks is no longer limited to model organisms.

In this chapter, we reviewed *Neurospora* as a potential model system for understanding the molecular mechanisms of clock variation and its consequences. Although *Neurospora* is a successful circadian model in eukaryotes (Dunlap & Loros, 2017), the molecular mechanisms of natural variation have not been fully explored. We hope this review will guide researchers to understand the importance of *Neurospora* in investigating the ecological importance of circadian clocks in fungi.

2. NEUROSPORA IN NATURE

2.1. The Genus of *Neurospora*

Red bread mold was first reported by Lveill in France in the 1840s. This fungal species was later incorporated into the genus *Monilia* as *Monilia sitophila*. By the early 20th century, this fungus had been identified across Europe and the USA. Experimental reports showed

that this fungus could grow in artificial cultures. In the late 1920s, based on the morphological (conidia and perithecia) similarities, some of the *Monilia sitophila* group species were transferred into the new genus *Neurospora* and named as *N. sitophila*, *N. crassa*, *N. tetrasperma* and *N. erythraea* (Shear & Dodge, 1927). Over the next few decades, several fungal species across the globe were studied and classified under the genus *Neurospora*. In the 1970s, Perkins and his colleagues collected over 4,500 *Neurospora* isolates from nature, analyzed their physiological characteristics, and classified them into groups: heterothallic (*N. crassa*, *N. discrete*, *N. intermedia* and *N. sitophila*), pseudohomothallic (*N. tetrasperma*) and homothallic (*N. africana*, *N. dodgei*, *N. galapagosensis*, *N. lineolate*, *N. terricola* and *N. pannonica*) (Perkins & Turner, 1988). *Neurospora* has three different types of spores; a sexual spore called an ascospore, and two asexual spores (also called conidia) called macroconidia and microconidia (Springer, 1993). Almost all non-homothallic species of *Neurospora* are conidiating species. (Turner, Perkins, & Fairfield, 2001). *Neurospora* species are also widely distributed geographically. Initially, *Neurospora* species were found only in tropical and sub-tropical regions, but were later identified across the globe. In the laboratory, one can produce hybrid populations by crossing *N. crassa* and *N. intermedia*, although the reproductive success rate is lower than that of a cross within species. This hybrid population has been a useful tool in testing ecological factors including reproductive isolation and speciation (J. R. Dettman, Anderson, & Kohn, 2008, 2010; J. R. Dettman, Jacobson, Turner, Pringle, & Taylor, 2003). Additionally, biological and phylogenetic species of the *Neurospora* genus have been well characterized by Dettman and his colleagues (J. R. Dettman, Harbinski, & Taylor, 2001; J. R. Dettman, Jacobson, & Taylor, 2003; Jeremy R. Dettman, Jacobson, & Taylor, 2006; J. R. Dettman, Jacobson, Turner, et al., 2003), which helps to understand clock variation within different ecotypes.

2.2. Natural Habitats

Ironically, the natural habitats of *Neurospora* are not well understood. Although there is vast amount of knowledge in *Neurospora* biology, most of the studies have been done in a controlled laboratory setting. There are few well-established ecological observations of *Neurospora*'s natural habitat. *Neurospora* is a fire-adapted fungus. The sexual spore is resistant to most environmental stresses and requires a heat shock or a furfural gas that is generated during the fire to germinate (Emerson, 1948; Goddard, 1935). This provides an advantage for local colonization of *Neurospora* after wildfires, as it has been shown that most of the natural strains of *Neurospora* are collected after wildfires (D. J. Jacobson et al., 2006). This suggests that *Neurospora* is not a competitive organism in the natural environment unless a condition is right; it cannot colonize on natural substrates unless other natural microbes are absent in a given experimental condition (Lee, 2012). The preferred natural habitat of *Neurospora* has also been shown to vary regionally. In North America, *Neurospora* prefer to colonize beneath tree bark, whereas in Europe they prefer to colonize above tree bark (D. J. Jacobson et al., 2006; David J. Jacobson et al., 2004).

2.3. Collection of *Neurospora* Ecotypes

There are more than 6,000 natural ecotypes that have been collected and deposited in the FGSC (Fungal Genetics Stock Center), including an informative record of the collection site

of each species deposited. Among the strains deposited in the FGSC, there were unique geographical collection sites for each of the three-major *Neurospora* species; *N. intermedia* (253 sites), *N. crassa* (114 sites), and *N. discreta* (85 sites) (Figure 1). The *N. discreta* species complex is a large clade of species that is different from the clade that includes *N. crassa*. Unlike other species of *Neurospora* whose habitat is mostly in temperate regions (Turner et al., 2001), *N. discreta* has successfully adapted to habitats in diverse latitudinal environments, and thus will likely be an ideal species for studying the local adaptation of circadian clocks (Figure 1).

2.4. *Neurospora* as a Model for Clock Variation Studies

Among all filamentous fungi, *Neurospora* has been widely used to understand several aspects of biochemistry, ecology, evolution, genetics, and circadian biology. *Neurospora* is a haploid filamentous fungus, which has a larger evolutionary timescale compared to other model systems (Baker, Loros, & Dunlap, 2012; Dunlap & Loros, 2017; Turner et al., 2001). *Neurospora* has been well adapted to different latitudes and longitudes across the globe. Therefore, it can potentially serve as a model for understanding several ecological and evolutionary aspects including natural variation. Around the 1970s, Perkins and his colleagues started collecting different species of *Neurospora* across the globe. Most of the collections were submitted to FGSC (Perkins & Turner, 1988; Perkins, Turner, & Barry, 1976; Turner et al., 2001). As mentioned in section 2.3, there are more than 6,000 *Neurospora* ecotypes deposited in FGSC. Among those collected by the FGSC, the most well-characterized reference species is *N. crassa*, the model species that has been widely used to understand the biological aspects of *Neurospora*. The genome of *Neurospora* is relatively small compared to other eukaryotes, and there is a strong synteny of genome among species, especially in *N. crassa*. Additionally, more than 11,000 knockout mutant strains were generated for understanding the gene functions and associated morphology (Colot et al., 2006; McCluskey, Wiest, & Plamann, 2010). In the laboratory, the maintenance of *Neurospora* is easy and economical. Since it has a short life cycle and can produce a high number of progeny by both asexual and sexual reproduction, it is a good model system for studying population genetics. The most documented circadian-regulated phenotype in *Neurospora* is asexual development, which is a fitness trait (Brandt, 1953; Pittendrigh, Bruce, Rosensweig, & Rubin, 1959). Based on multiple advantages, *Neurospora* can serve as a potential model to understand natural variations of the circadian clock and its fitness.

3. THE CIRCADIAN CLOCK OF *NEUROSPORA*

Our intention in this section is not an in-depth review of all known molecular mechanisms of the *Neurospora* circadian clock, but rather a brief review to understand studies in natural variation of the *Neurospora* clock. We recommend to readers other excellent reviews on *Neurospora* circadian clocks for a more comprehensive description (Dunlap & Loros, 2017; C. Heintzen & Liu, 2007; J. Hurley, Loros, & Dunlap, 2015; Lakin-Thomas, Bell-Pedersen, & Brody, 2011).

3.1. FRQ Dependent Oscillators

Although *Neurospora* is a simple eukaryotic organism in comparison to higher eukaryotes, the molecular structure of the *Neurospora* circadian clock shows significant complexity, thus providing an opportunity to dissect network motifs for more complex eukaryotic circadian systems (Bell-Pedersen, Crosthwaite, Lakin-Thomas, Merrow, & Okland, 2001). This molecular structure is composed of the negative components FREQUENCY (FRQ) and VIVID (VVD), and the positive components WHITE COLLAR-1 (WC-1), and WHITE COLLAR-2 (WC-2). (Baker et al., 2012; P. Cheng, Y. Yang, K. H. Gardner, & Y. Liu, 2002; Dunlap & Loros, 2017; J. Hurley et al., 2015). Traditionally, clock studies were aimed at studying three aspects of the clock: input, the oscillator, and output. In *Neurospora*, FRQ is a key component of the oscillator, WC-1 and WC-2 are part of both the input and the oscillator, and VIVID (VVD) is part of both the input and the output (Q. He & Liu, 2005a; Schneider et al., 2009; Zoltowski & Crane, 2008). It has been shown that WC-1 is a blue light receptor for the circadian oscillator (Allan C. Froehlich, Liu, Loros, & Dunlap, 2002; Qiyang He et al., 2002), and FRQ-interacting RNA Helicase (FRH) is a protein which interacts with the clock component FRQ (Ping Cheng, Qun He, Qiyang He, Lixin Wang, & Yi Liu, 2005). Several studies have reported the role of casein kinases 1 and 2 (CK1 and CK2) in the negative feedback loop (Q. He, Cha, He, et al., 2006; Mehra et al., 2009; Querfurth et al., 2011). The key positive elements in the feedback loop contain PAS domain(s), through which they form homo- or hetero-dimers. This includes VVD, another PAS protein and a blue light receptor, which was shown to form a second connected negative feedback loop through WC-1 (Christian Heintzen, Loros, & Dunlap, 2001; Schwerdtfeger & Linden, 2003). The emerging picture is that the clock is composed of multiple regulatory loops.

3.1.1. Simple and Coupled Loops—Positive regulation of WC-1: WC-1 is a blue light receptor and a transcription binding factor (Allan C. Froehlich et al., 2002; Qiyang He et al., 2002), and regulates its own expression (Ballario et al., 1996; P. Cheng, Yang, Wang, He, & Liu, 2003). When either of the functional WC-1 or WC-2 proteins are absent in a cell, *wc-1* transcription is not light induced (Ballario et al., 1996). Later, it was shown that WC-1 and WC-2 work as a heterodimer activator complex (White Collar Complex, WCC) (Ping Cheng, He, Yang, Wang, & Liu, 2003; Ping Cheng, Yuhong Yang, Kevin H. Gardner, & Yi Liu, 2002; Allan C. Froehlich et al., 2002; Qiyang He et al., 2002; Talora, Franchi, Linden, Ballario, & Macino, 1999). Although at the surface WCC seems to have a simple genetic regulation with the forward regulation feedback loop, there is more complex regulation at the molecular level.

Coupled loops in WCC: WC-1 negatively regulates WC-2 expression (P. Cheng et al., 2003), and thus, forms a coupled loop within WCC regulation. WC-1 is a multi-functional protein that contains motifs for sensing the environment and protein-protein interactions, and activation domains with specialized roles in the light and darkness (P. Cheng et al., 2003; Lee, Dunlap, & Loros, 2003; Toyota, Onai, & Nakashima, 2002). Expression of *wc-1* appears to be regulated for its multiple functions, with three promoters identified as necessary for the transcription of *wc-1* (Kaldi, Gonzalez, & Brunner, 2006). One of the promoters, P_{dist} , is responsible for the expression of WC-1 in darkness, and its transcription

activity is dependent on WC-1 function. The transcriptional activity of another promoter, P_{prox} , is independent of WC-1 in the dark. The last promoter, P_{int} , is located in the open reading frame of the *wc-1* gene. The transcription activity of the promoter P_{int} is insensitive to a light signal. The alternative translational start site in the P_{int} -originated transcript produces a truncated WC-1 protein that is lacking the amino-terminal activation domain, NpolyQ, whose activation function influences the circadian period of an organism (Lee et al., 2003; Michael et al., 2007; Toyota et al., 2002). The altered transcriptional activity of *wc-1* also leads to an altered phase of an organism (Kaldi et al., 2006). It appears that WCC can regulate WC-1 expression by choosing different regulatory sites depending on ambient light conditions.

Negative regulation of FRQ: One of the major conceptual breakthroughs in circadian clock studies was the demonstration of the negative feedback loop of the negative regulator, FRQ (Aronson, Johnson, Loros, & Dunlap, 1994). In this classical study, the authors used an overexpression construct of FRQ. When FRQ was overexpressed in a cell by an inducer, its own transcription was down-regulated. The detailed mechanisms of the negative feedback mechanisms, however, have been shown to be more than simple negative regulation. Constitutive expression of *qrf* (*frequency antisense transcript*) negatively regulated the level of *frq*, and the absence of *qrf* compromises the light dependent resetting of clock (Li, Joska, Ruesch, Coster, & Belden, 2015; Xue et al., 2014).

3.1.2. Interlocked Loops in WCC/FRQ—WCC and FRQ control each other's expression through coupled positive and negative regulatory loops.

Negative feedback loop: The WCC binds to the proximal light regulatory element (PLRE) and clock-responsive element (C-box) in the *frq* promoter. Binding of the WCC to the PLRE leads to acute *frq* induction upon light exposure, which resets the circadian clock. Binding of the WCC to the C-box activates the transcription of *frq*, which is involved in the transcriptional/translational feedback loop of the core circadian oscillator. However, binding of the WCC to the C-box can be interrupted by nucleosomes. SWI-SNF chromatin remodeling complex clears nucleosomes and promotes the binding of the WCC to the C-box and induces the expression of *frq* (Dunlap & Loros, 2017; Wang, Kettenbach, Gerber, Loros, & Dunlap, 2014). After the *frq* gene is translated, FRQ homodimers bind to FRH (FRQ-associated RNA helicase) in the cytoplasm and form the FFC (Negative elements), which enhances the stability of FRQ (P. Cheng, Q. He, Q. He, L. Wang, & Y. Liu, 2005; Guo, Cheng, & Liu, 2010). Upon localization of FFC into the nucleus, several kinases interact with the FFC complex to phosphorylate FRQ (Baker et al., 2012; J. Hurley et al., 2015). Of these kinases, casein kinases (CK1a and CK2) are particularly associated with clock dependent phosphorylation (Q. He, Cha, He, et al., 2006; Mehra et al., 2009; Querfurth et al., 2011). CK1a interacts with FFC through the FRQ/CK1a interaction domain (FCD), which is located near the three phosphorylation sites of the *frq* gene. The interaction of CK1a with the FFC plays a crucial role in the negative feedback mechanism. CK1a also phosphorylates and inactivates the WCC. This process is mediated by the physical interaction between the FFC and the WCC (Q. He, Cha, He, et al., 2006). As the activator function of the WCC diminishes, the level of *frq* transcription also diminishes. After the rate

of *frq* transcription and translation slows down, the level of FRQ in the nucleus diminishes (Aronson et al., 1994; Q. He, Cheng, Yang, Yu, & Liu, 2003). The low level of FRQ in the nucleus allows WCC in the nucleus to resume transcription of *frq*, which closes the negative feedback loop. The period of circadian oscillation of the clock gene FRQ is about 22 h, which includes the time for synthesis, phosphorylation, and degradation of the FRQ protein (Dunlap & Loros, 2017; J. Hurley et al., 2015; M. W. Merrow, Garceau, & Dunlap, 1997).

Positive feedback loop: FRQ is involved not only in negative regulation of its activators (WCC), but also in the positive regulation of WCC. FRQ is involved in the up-regulation of WC-1 at the post-transcriptional level and in the up-regulation of WC-2 at the transcriptional level (P. Cheng, Yang, & Liu, 2001; Lee, Loros, & Dunlap, 2000). Thus, FRQ plays a role in coupling the negative and positive loops. This inter-connected loop has also been reported in *Drosophila* and mammals (Glossop, Lyons, & Hardin, 1999; Shearman et al., 2000). In all cases, the inter-connected loop plays a crucial role in maintaining the robustness of the circadian oscillation (P. Cheng et al., 2001).

3.1.3. Post-Translational Modification of WCC and FFC—In addition to transcriptional/translational feedback regulation, post-translational regulation plays a role in circadian regulation (Brunner & Schafmeier, 2006). The phosphorylation status of the WCC is important for its activation and functionality. Phosphorylation of the WCC is FRQ-dependent, and hyper-phosphorylated WCC is an inactive form of WCC (Q. He & Liu, 2005b; Schafmeier et al., 2005). Upon exposure to light, the WCC forms a complex that is much larger than the WCC in darkness (Allan C. Froehlich et al., 2002; Q. He & Liu, 2005b). Because the number of FFCs in a cell is lower than the number of WCCs, it was speculated that the negative function of FRQ-FRH could be achieved by recruiting kinases for phosphorylation of the WCC. There are several kinases that are known to phosphorylate clock proteins, (Ping Cheng et al., 2005; A. C. Froehlich, Noh, Vierstra, Loros, & Dunlap, 2005; Görl et al., 2001; Q. He, Cha, Lee, Yang, & Liu, 2006) including casein kinases, which phosphorylate the WCC and FRQ. To finish the negative loop, the negative elements are degraded. This is achieved through the ubiquitin-proteasome system much like in mammals and *Drosophila* (Q. He & Liu, 2005a). There are four kinases and two phosphatases involved in FRQ phosphorylation (Garceau, Liu, Loros, & Dunlap, 1997; Görl et al., 2001; Liu, Loros, & Dunlap, 2000; Pregueiro, Liu, Baker, Dunlap, & Loros, 2006; Yang, Cheng, & Liu, 2002; Yang, Cheng, Zhi, & Liu, 2001; Yang et al., 2004). Eventually, hyper-phosphorylated FRQ is recruited by FWD-1, an F-box/WD-40 repeat-containing protein. FWD-1, a substrate-recruiting subunit of an SCF (SKP/Cullin/F-box)-type ubiquitin ligase, physically interacts with hyper-phosphorylated FRQ, which leads to the degradation of the FRQ protein (Q. He et al., 2003; Q. He & Liu, 2005a). For the simplicity of the model, we did not include the degradation of FRQ by ubiquitination in our model. In the absence of FRQ, FRH can interact with WCC (Guo et al., 2010), however, the role of FRH in the circadian clock was unclear. It has been shown that FRH also plays a vital role in cell viability (J. M. Hurley, Larrondo, Loros, & Dunlap, 2013; Lauinger, Diernfellner, Falk, & Brunner, 2014; Shi, Collett, Loros, & Dunlap, 2010), though a recent study has shown that FRH may not play an essential role in the circadian clock (Conrad et al., 2016).

3.1.4. Negative Feedback Loop in WCC/FRQ/VVD—The gene VVD also forms negative feedback loops. VVD is another blue light receptor that has two major roles: sensing the change of light intensities and photoadaptation (Q. He & Liu, 2005b; Christian Heintzen et al., 2001; Schwerdtfeger & Linden, 2003; Shrode, Lewis, White, Bell-Pedersen, & Ebbole, 2001). The role of VVD in endogenous clock regulation is that of a clock-controlled output gene, and also a modulator of the light input pathway to the clock (Chen, DeMay, Gladfelter, Dunlap, & Loros, 2010; Christian Heintzen et al., 2001; Hunt, Thompson, Elvin, & Heintzen, 2010). VVD is one of the early light-response genes that are activated by WCC (Q. He & Liu, 2005b; Christian Heintzen et al., 2001; Schwerdtfeger & Linden, 2003). Once VVD is expressed, it negatively regulates its own expression by blocking the activator function of the WCC by directly binding to the WCC (Chen et al., 2010; Hunt et al., 2010). This photo-adaptation function ensures the circadian oscillator runs during the daytime (Elvin, Loros, Dunlap, & Heintzen, 2005). VVD also plays a key role in resetting circadian rhythm at dusk (light to dark transition) by enhancing *frq* degradation, and thus, *de novo* synthesis occurs, causing a new cycle to begin (Elvin et al., 2005).

3.2. FRQ-Independent Oscillators

In the 1980s, Feldman's lab first reported FLO (FRQ less oscillator) rhythms using a *frq* mutant (Loros, Richman, & Feldman, 1986). Over the years, several studies were done to understand the mechanism underlying the developmental rhythms by FLO. In the late 90s, a report was published that *frq⁹* mutants can be entrained by temperature, but not with light (Chang & Nakashima, 1997; M. Merrow, Brunner, & Roenneberg, 1999). Furthermore, Lakin-Thomas and Brody published that deficiency of lipids can restore the developmental rhythms in absence of a core circadian oscillator, which includes photoreceptors like *wc-1* and *wc-2* (Lakin-Thomas & Brody, 2000). These observations support that temperature and metabolism can play an essential role in generating developmental rhythms in addition to the core circadian oscillator. Based on these studies, a two-oscillator model has been proposed; the core circadian oscillator responds to light and FLO responds to non-photic signals such as temperature. These two oscillators may or may not be coupled to generate developmental rhythms (Bell-Pedersen et al., 2001). In addition to a Choline-Deficient Oscillator, CDO (Lakin-Thomas, 1998; Lakin-Thomas & Brody, 2000), a Farnesol or Geraniol based Oscillator, FGO (Granshaw, Tsukamoto, & Brody, 2003), and a Cryptochrome-dependent Oscillator, CRO (Nsa et al., 2015) have been identified in *N. crassa*. Additionally, microarray studies have shown that several clock-controlled genes are rhythmic in the absence of *frq* (Correa et al., 2003). This suggests that different FLOs can take over the developmental rhythms. The relationships between the FRQ-based oscillator and FLOs have yet to be characterized.

In the context of natural variation, metabolic enzymes and metabolic components, e.g. mitochondria, have been reported (Table 1). Interestingly, allelic variations of *nit-2* for nitrate reductase and *nit-4* for nitrogen regulation have been observed in *N. sitophila* and *N. intermedia* respectively. Recently, a study has been done on the *frq*-less Nitrate Reductase Oscillator in *N. crassa*, NRO (Christensen et al., 2004), in which NIT-2 and NIT4 are the positive elements of the feedback loop.

4. NATURAL VARIATION AND THE CIRCADIAN CLOCK

There is a large volume of literature on natural variation studies using *Neurospora*. In section 4.1., we will highlight a few selected studies that are worth revisiting in the study of natural variation of the *Neurospora* circadian clock. Considering the recent advancements in our understanding of the existence of metabolic oscillations and their cross-talk with the genetic oscillator, we believe that old literature on natural variation studies in metabolites and enzymes can provide potentially new insights for studying natural variation in *Neurospora* circadian clocks.

4.1. Natural Variation in *Neurospora* Ecotypes

From the 1920s, *Neurospora* wild isolates from different natural habitats were grown in synthetic media to study physiological variation of ascospores, conidia, and perithecia (female sexual organ). The ability to grow *Neurospora* in a defined artificial media made *Neurospora* an attractive model organism. Different species of *Neurospora* have been characterized based on physiological variations such as number of asci, ascospore size, pigmentation, the size of perithecia, mating behavior, etc. (Perkins et al., 1976; Shear & Dodge, 1927; Tai, 1935). Several early studies have shown phenotypic variation in different environmental conditions: speed of conidial germination was influenced by the ambient temperature (Shear & Dodge, 1927; Thom & Ayers, 1916), a higher ascus abortion rate in the Texas strain of *N. tetrasperma* has been reported on different media (Dodge, 1939), and mycelial growth and sexual development of *N. lineolate* varied in different media conditions (Frederick, Uecker, & Benjamin, 1969). Other investigations on nutritional factors suggest that variations in perithecia formation occur due to the concentrations of glucose and potassium nitrate of a synthetic medium, which favors sexual reproduction in *Neurospora* (Westergaard & Mitchell, 1947). Around the 1950s, scientists started to understand the biochemistry of conidia and mycelia using *Neurospora*. A 1954 study reported the importance of light and oxygen in the synthesis of carotenoids of *N. crassa* (Zalokar, 1954). Furthermore, variations in respiratory enzymes such as NAD nucleotidase, β -galactosidase and β -glucosidase have been reported in conidia compared to the mycelia (Eberhart, 1961; Zalokar, 1959; Zalokar & Cochrane, 1956). The activity of the citric acid cycle was shown to decrease in conidia. Therefore, Weiss and his colleagues began to understand the variation in activity of mitochondrial enzymes such as oxidative and glycolytic enzymes. Among them, variation of glycolytic enzymes (ethanol dehydrogenase and pyruvate carboxylase) has been reported in conidial and mycelial cultures (Weiss & Turian, 1966).

Several studies have also investigated the genetic control associated with phenotypic variations of enzymes in wild isolates of *Neurospora*. *ars* for aryl-sulfatase (Metzenberg & Ahlgren, 1971); *arg-12* for ornithine transcarbamylase (Grindle & Davis, 1970); *leu-5* for leucyl-tRNA synthetase (Beauchamp, Horn, & Gross, 1977); *inv* for invertase (Yu, Garrett, & Sussman, 1971); *nit-4* for nitrate reductase (Perkins & Turner, 1988); *pts-1* for protease (Hanson & Marzluf, 1975); *nit-2*, *nmr* for nitrogen regulation (Grove & Marzluf, 1981; Young & Marzluf, 1991) and *T* locus for tyrosinase (Horowitz, Fling, Macleod, & Sueoka, 1961; Ruegg, Ammer, & Lerch, 1982). Several other studies have investigated the variation in other enzymes such as amylases, allozymes, β -glucosidases, cellulases, and esterases, etc

(Perkins & Turner, 1988; Perkins et al., 1976; Reddy & Threlkeld, 1971a, 1971b, 1972; Spieth, 1975; Turner et al., 2001). Interestingly, most of the enzymatic variations have been associated with enzymes involved in metabolic pathways. In addition to enzymatic variations, mitochondrial variants have been reported in natural populations of *Neurospora*.

In the late 1970s, mitochondrial DNA variants were reported in *N. crassa* (Mannella, Pittenger, & Lambowitz, 1979). Later, mitochondrial DNA variants were identified in inter and intraspecific populations of *Neurospora* (Collins & Lambowitz, 1983; Griffiths, 1995; Perkins & Turner, 1988; Taylor, 1986; Taylor, Koupel, & Whitmer, 1987; Turner et al., 2001). Additionally, natural variation in the shape and size of mitochondrial plasmids was found in different species of *Neurospora*. Integration of circular plasmids into the mitochondrial genome can interfere with mitochondrial functions, subsequently causing death of cultures (Senescence), which has been observed mainly in *N. intermedia* (Griffiths, 1992, 1995; Maheshwari & Navaraj, 2008; Perkins & Turner, 1988; Turner et al., 2001). Table 1 summarizes more natural variation studies, which might be useful for characterizing the natural variation in circadian clock. For more information on natural variations and *Neurospora* natural population, there are excellent reviews available (Perkins & Turner, 1988; Perkins et al., 1976; Turner et al., 2001).

4.2. Natural Variation of Core Clock Genes

Using inverted race tube assays (Park & Lee, 2004), 143 ecotypes of *N. crassa* have been characterized. There was significant variation in circadian period, phase, and temperature compensation among the ecotypes (Michael et al., 2007). In addition to the phenotypic variation of the clock, genetic variation has been studied in four key clock genes, FRQ, WC-1, and WC-2, and VVD. A subsequent study tested the hypothesis that the copy number variation of Simple Sequence Repeats (SSR) in the clock genes could serve as a way of creating phenotypic variation in *Neurospora*. This hypothesis was based on one of the conclusions from a parallel genome-wide study of the SSR distribution in *Neurospora* genomes that tri-nucleotide SSRs in the exon might play a role in the variation of protein functions in *N. crassa* (Kim et al., 2008). All core clock genes contain SSR repeats. Most interestingly, a photoreceptor and transcription factor WC-1 has two large SSR repeats: one in the amino-terminal, NpolyQ, and one in the carboxyl-terminal, CpolyQH. In the *wc-1* alleles that lack NpolyQ, *wc-1^{MK1}* and *wc-1^{thy-2}*, there is no functional clock running, whereas, *frq* is light induced (P. Cheng et al., 2003; Lee et al., 2003; Toyota et al., 2002). This suggests that WC-1 activation domains may function in a modular fashion, and NpolyQ could be a main activation domain for the clock function. The estimated repeat size of NpolyQ was between 17–80 base pairs among ecotypes, and the laboratory strain 87-3 had 39 repeats. There was a significant correlation between the size of NpolyQ repeats and the circadian period among 143 natural ecotypes. To test if the size of NpolyQ repeats explains the period variation, two ecotypes with similar structural features except the size of NpolyQ repeats were crossed: FGSC#3223 (29 repeats) and FGSC#4724 (57 repeats). The period of F1 progeny segregated with the size of NpolyQ alleles. The authors concluded that the natural variation in WC-1 NpolyQ of ecotypes may have served for fine tuning their circadian clock to a local habitat (Michael et al., 2007). This suggests that SSR variation could be the answer to how new gene function could arise in a genome with several strong

genome defense mechanisms in which any duplicated genome sequences more than 500 nt would be mutated (Aramayo & Selker, 2013).

The conservation of FRQ function in the family Sordariomycetes has been demonstrated by complementing the *Neurospora frq* mutant by expressing the FRQ homolog from *Sordaria fimicola*. The resulting fusion protein had 11 amino acids from *N. crassa* in the amino-terminal with the rest of protein sequence from *S. fimicola* (M. W. Merrow & Dunlap, 1994). Encouraged by this success, the sequence of the *frq* gene has been used to establish a phylogenetic relationship among species in the genus of *Neurospora* and in Ascomycete fungi with a limited success (Lewis & Feldman, 1996; Skupski, Jackson, & Natvig, 1997). A more comprehensive sequence analysis has been done with five clock genes (FRQ, WC-1, WC-2, FRH-1, and FWD-1) in 64 sequenced fungal genomes (Salichos & Rokas, 2010). FRH-1 and FWD-1 appeared to be the most ancient proteins, followed by WC-1 and WC-2. Interestingly, FRQ appeared to be the most recent protein and appears in only three families: Sordariomycetes, Leotiomycetes, and Dothideomycetes in the Ascomycetes (Salichos & Rokas, 2010).

4.3. Technical Challenges for Studying Clock Variation in *Neurospora* Natural Populations

As is true for other biological research, having an easily assayable phenotype is important for a successful outcome. For *Neurospora* circadian studies, the rhythmic asexual development (conidiation) was a blessing. In the 1950s, the rhythmic pattern of conidiation in *Neurospora* was reported (Brandt, 1953; Pittendrigh et al., 1959). Unlike the unicellular yeast, filamentous fungi like *Neurospora* will grow continuously away from the center of inoculation. Spending the majority of time in the vegetative stage, *Neurospora* will grow as mycelia (transparent or white color). Once approximately 24 hr have passed, *Neurospora* will stop growing as mycelia and start producing conidia (orange color). The conidia are produced from a specialized aerial hyphae cell type called a conidiophore (Springer & Yanofsky, 1989). In a macroscopic view, the area of *Neurospora* that produces conidia is seen as denser in comparison to that of mycelia. This dense area is called a 'band'. When *Neurospora* is grown in a glass tube with a medium on the bottom (race tube), the developmental pattern can be easily visualized. To measure the circadian period (in constant darkness condition), or phase (in a cyclic light/dark condition), the growing front of the mycelia is marked on the glass tube under the red light, which does not interfere the *Neurospora* circadian rhythm. Period is measured by the distance from one reference point to the next. Phase is measured as the distance from a reference point of environmental cycle, e.g. dark to light transition, or to a reference point of a development. Traditionally, the reference point has been the peak of a conidiation band. Recently, the photographic image is read by software such as Chrono, developed by Till Roenneberg, to calculate the clock phenotypes of the sample using different reference points, e.g. trough, onset or offset (Roenneberg & Taylor, 2000). However, visualizing the developmental bands in the natural strains was not a trivial matter. Conidiation is repressed by the accumulated CO₂ produced by the respiration of the cells, which is trapped inside of the race tube (Sargent & Kaltenborn, 1972). Therefore, virtually all circadian studies in *Neurospora* have been done in the band (*bd*) mutant background to visualize the banding phenotype (Sargent & Woodward, 1969). The *bd* mutation enabled the fungus to overcome the adverse effect of

CO₂ accumulation on conidiation. Four decades after the discovery of the band mutation, it was shown to be located in the *ras-1* gene, which is involved in producing reactive oxygen species known to stimulate conidiation (Belden et al., 2007). Unfortunately, one cannot use this mutant background to study circadian rhythms of ecotypes. To create the *bd* mutant background, one has to cross an ecotype with a *bd* mutant. In this process, the unique genotype adapted to one local habitat will be lost. Alternative methods to study the ecotypes without crossing them to a *bd* mutant strain were proposed, such as blowing fresh air to remove CO₂ or adding Rubidium-chloride to the medium (Morgan & Feldman, 1998). These methods were not ideal, however, as blowing air into a race tube tends to spread the hydrophobic conidia into other parts of the media, and adding RuCl causes additional variables in an asexual development of wild ecotypes. One successful assay method to visualize the overt developmental rhythm of natural ecotypes was developed by simply inverting the conventional race tubes. This is known as an inverted race tube assay (Park & Lee, 2004). This modified race tube assay allowed the accumulated CO₂ in the race tube to be ventilated out without blowing fresh air into the race tube, as CO₂ is heavier than air. Because the natural strains grow faster than the band mutants, as the *bd* mutation reduces growth rate, it was necessary for the modified race tube to be longer than the conventional race tube designed for the *bd* mutants (Park & Lee, 2004).

4.4. Methods for Clock Variation Studies

4.4.1. QTL Analysis on Clock Phenotypes—To elucidate the mysteries of gene function caused by genetic variations in the natural population, quantitative genetic techniques have been successfully utilized over the past decades (Alonso-Blanco & Koornneef, 2000). These methods describe how known mutant loci can interact with one another, as well as to isolate new loci in the same pathway (Shimomura et al., 2001). Over the past century, the field of quantitative genetics has grown out of the desire to exploit natural variation and to describe traits with a continuous distribution of phenotypes. An extension of Mendelian principles of polygenic trait is quantitative genetics (phenotypes encoded by multiple loci). Most of the phenotypic variation identified in natural populations is due to quantitative trait loci (QTL), in which multiple loci contribute small effects on phenotypes, as opposed to a single major-effect locus (Alonso-Blanco & Koornneef, 2000).

Haploid organisms have multiple advantages for studying quantitative traits: 1) For assays, a large number of individuals can be maintained clonally, which may reduce the error from environment; 2) they have a single allele at any given locus, so dominance does not contribute to the genetic variation; 3) QTL can be detected after the first generation; and, as in the case of *Neurospora*, 4) they are fast and easy to work with. For these reasons, *Neurospora* can serve as a potential model to elucidate fundamental questions of quantitative genetics for complex behaviors including circadian clocks. As our understanding of the molecular structure of circadian clocks advances, it becomes apparent that the circadian clock is more tightly linked with other cellular machinery than we imagined (Bell-Pedersen et al., 2001). Based on this, one cannot dissociate the circadian clock from basic cellular metabolism. In previous efforts to find clock genes, all morphological mutants and any mutants with abnormal metabolisms other than the period phenotypes were excluded from analysis. As we have learned more about the importance of the circadian clock in cellular

activities, we have realized that there could be clock genes involved in these basic metabolic activities as well. These genetic loci with subtle clock phenotypes or with essential cellular functions could not be studied using traditional genetic screening methods. QTL analysis could reveal novel clock genes and clock mechanisms which would be overlooked by the traditional forward genetics approaches. QTL analysis is a systematic way of mapping all the multiple genetic loci for subtle quantitative traits and essential cellular functions.

To study the natural allelic variants, three mapping populations were prepared using six *N. crassa* ecotypes from distant geographic locations (N2; FGSC3223 from USA x FGSC4724 Malaysia, N4; FGSC4720 from India x FGSC4715 Haiti, N6; FGSC4825 Ivory Coast x FGSC2223 USA). QTL analysis on circadian period and entrained phase phenotype identified 43 QTL (Kim, Logsdon, Park, Mezey, & Lee, 2007). Thirteen of 43 QTL co-localized to known clock genes, which supports that QTL analyses can identify clock genes. More surprising was that 30 of 43 QTL (70%) did not co-localized to known clock genes. This suggests that there are many uncharacterized genetic elements whose variation contributed to the variation of the circadian clock for a specific habitat. Identifying QTL (a chromosomal region which could contain multiple genetic elements) is relatively easy in comparison to identifying causative genes and the nature of variation that caused the phenotypic variation. Since *Neurospora* has a relatively small haploid genome (about 40 Mb), it is a promising system to characterize the causative natural allelic variation for clock variation.

4.4.2. Circadian Clocks in Natural Substrates and Natural Environment—Unlike animals, a filamentous fungus cannot move to other locations when one place does not provide a favorable environment. However, a filamentous fungus can grow indefinitely to a new place that might provide a better living condition. This is why filamentous fungi are the largest living organisms on earth. In one recorded case, one genetically identical fungus *Armillaria bulbosa* grew over 1,500 years covering over 15 hectares with an estimated weight over 10 tons (M. L. Smith, Bruhn, & Anderson, 1992). One might have a hard time justifying ecological studies on circadian rhythms of *A. bulbosa* in a 27-inch race tube under the laboratory conditions. For the reasons that we argued throughout this review, *Neurospora* is the best model system for ecological and evolutionary studies of circadian rhythms. The current challenge is developing an experimental system that utilizes natural substrates in a natural environment, and generates reproducible experimental data for characterizing circadian rhythms of a fungal strain. As the first step toward this goal, *Neurospora* has been grown on different natural substrata (Lee, 2012). Utilizing new functional genomics tools, *Neurospora* could serve as a model system for addressing circadian-clock associated ecological questions (Ellison et al., 2011; Lee, 2012; Lee & Dighten, 2013; Romero-Olivares, Taylor, & Treseder, 2015)

5. Future Challenges

Considering the important ecological role of the fungal kingdom in the ecosystem on earth, we have no doubt that understanding the natural variation of the fungal circadian clock is both interesting and an important area of research. As discussed earlier, we are hopeful that more researchers will invest time in the study of natural variation of fungal circadian clocks

due to the many advantages that the system offers. However, we also expect a few tough challenges that require much deliberation and a consensus from the research community. These challenges are present not only in the study of fungal clocks, but also in natural variation studies in general.

There is a great advantage in working with a model organism like *Neurospora*, especially when it is a reference strain with a great deal of resources. Because of the large amount of resources invested on a few select model organisms, we now enjoy a detailed understanding of clock mechanisms. In choosing a model reference organism, it was necessary that the three minimum characteristics required to be called an ‘authentic’ circadian clock be present: an approximate 24 hr period in constant conditions, the oscillator is entrainable by the local environment, and period is temperature compensated within a physiological range of temperatures. However, as we study the ecotypes (or accessions) of the model organisms that are adapted to a diverse habitat, we find significant deviations in these clocks from that of the reference strain. Circadian clocks have been observed in fungi other than *N. crassa*, however, these clocks are not satisfying the community-agreed criteria to be called an authentic circadian clock (Austin, 1968; Bluhm, Burnham, & Dunkle, 2010; Greene, Keller, Haas, & Bell-Pedersen, 2003; Hevia, Canessa, & Larrondo, 2016; Hevia, Canessa, Muller-Esparza, & Larrondo, 2015; Oliveira et al., 2015; Traeger & Nowrousian, 2015). We believe it is more interesting to find different kinds of clocks with different properties, which reflect the life history of the organism (or species) and speak volumes about the real biology of the clock in nature. As we classify circadian clocks into ‘authentic’ and ‘not-so-authentic’, we may somehow be de-emphasizing the studies of those clocks that are not satisfying the minimum criteria of the authentic clock.

Most of the known clock properties in a model organism have been characterized in a controlled laboratory environment using laboratory strains, which served its purpose well to generate reproducible data. However, to study the ecological roles of the circadian clocks, we need to perform experiments in their natural habitat. In a pleasant spring day, the surface temperature of the ground (where many fungi live) could fluctuate from 0 – 40° (Celsius) in a day. The physiological temperature range that we know for temperature compensation spans 20 – 30° for a typical laboratory *Neurospora* strain. We envision a need to develop criteria to characterize non-model fungal clocks (or ecotypes of model species) in natural habitats.

Several studies on eukaryotes shown the role of circadian clock in maintaining fitness (Beaver et al., 2002; Gill & Panda, 2011; Green, Tingay, Wang, & Tobin, 2002; Miller et al., 2004; Xu, DiAngelo, Hughes, Hogenesch, & Sehgal, 2011). Even though, asexual reproduction is a fitness trait of filamentous fungi like *Neurospora*, natural variations of the circadian clock and associated fitness trait is not well understood. There are different ways of measuring ‘fitness’ in filamentous fungi, but there is no clear consensus in the fungal community. One could measure the growth rate, estimating the amount of nuclei by PCR, number of conidia, germination rate of conidia, number of perithecia (fertilized female organ), number of ascospores (sexual spore), germination of ascospores, etc (Pringle & Taylor, 2002). According to a classical definition of fitness, one might measure the number of progeny (conidia or ascospore). However, there are many aconidial species that do not

produce conidia. Even in conidial species with a known sexual cycle, the number of progeny is greatly changed depending on the media and environment. Many fungal species also have different developmental pathways. For example, *Neurospora* has two different asexual progeny (macroconidia and microconidia) and one sexual progeny (ascospore). Macroconidia contain 3–5 nuclei, and microconidia contain one nucleus. Depending on the natural habitat a *Neurospora* strain lands on, it will produce different ratios of macroconidia to microconidia (Lee, 2012). It is a challenging task to measure fitness in an unbiased way. We hope that it will prove possible to develop a consensus on what is an appropriate measure of fitness.

Technical Challenges: In an ideal situation, one would like to study a natural strain in a natural habitat. Unlike animals or plants, it is difficult to study fungi in their natural environment. A luciferase reporter is a great way of monitoring the molecular clock, assuming that the fungus of interest can be transformed with the reporter construct and have its expression measured by a luminometer. One possibility to remedy this difficulty is to create a laboratory setting that closely mimics the natural environment.

CONCLUDING REMARKS

Even though *Neurospora* is a classical model for studying the circadian clock, it has not been widely used for understanding the ecological and evolutionary aspects of clocks. In this chapter, we reviewed the challenges, advantages, and promising future of the system to study natural variation of circadian clocks. We proposed that *N. discreta* be used for studying effects on fitness, as it has greater adaptability to different latitudinal environments compared to other *Neurospora* species (Figure 1). Since asexual development is one of the most well documented clock-regulated phenotypes, *Neurospora* is an excellent system to study the evolutionary and ecological roles of natural variation of the circadian clock (Figure 2).

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References

- Adamsson M, Laike T, Morita T. Annual variation in daily light exposure and circadian change of melatonin and cortisol concentrations at a northern latitude with large seasonal differences in photoperiod length. *J Physiol Anthropol.* 2016; 36(1):6.doi: 10.1186/s40101-016-0103-9 [PubMed: 27435153]
- Al-Saqur A, Smith BR. Surfactant resistance in natural *Neurospora* populations. *Neurospora Newsl.* 1980; (27):17.
- Allebrandt KV, Roenneberg T. The search for circadian clock components in humans: new perspectives for association studies. *Braz J Med Biol Res.* 2008; 41(8):716–721. [PubMed: 18797707]
- Alonso-Blanco C, Koornneef M. Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. *Trends Plant Sci.* 2000; 5(1):22–29. [PubMed: 10637658]
- Aramayo R, Selker EU. *Neurospora crassa*, a Model System for Epigenetics Research. *Cold Spring Harbor Perspectives in Biology.* 2013; 5(10)doi: 10.1101/cshperspect.a017921

- Aronson BD, Johnson KA, Loros JJ, Dunlap JC. Negative feedback defining a circadian clock: autoregulation of the clock gene *frequency*. *Science*. 1994; 263:1578–1584. [PubMed: 8128244]
- Austin B. An Endogenous Rhythm of Spore Discharge in *Sordaria fimicola*. *Annals of Botany*. 1968; 32(2):262–278. DOI: 10.1093/oxfordjournals.aob.a084207
- Baker CL, Loros JJ, Dunlap JC. The circadian clock of *Neurospora crassa*. *FEMS Microbiol Rev*. 2012; 36(1):95–110. DOI: 10.1111/j.1574-6976.2011.00288.x [PubMed: 21707668]
- Ballario P, Vittorioso P, Magrelli A, Talora C, Cabibbo A, Macino G. White collar-1, a central regulator of blue light responses in *Neurospora*, is a zinc finger protein. *EMBO J*. 1996; 15(7): 1650–1657. [PubMed: 8612589]
- Beauchamp PM, Horn EW, Gross SR. Proposed involvement of an internal promoter in regulation and synthesis of mitochondrial and cytoplasmic leucyl-tRNA synthetases of *Neurospora*. *Proc Natl Acad Sci U S A*. 1977; 74(3):1172–1176. [PubMed: 139609]
- Beaver LM, Gvakharia BO, Vollintine TS, Hege DM, Stanewsky R, Giebultowicz JM. Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. *Proc Natl Acad Sci U S A*. 2002; 99(4):2134–2139. DOI: 10.1073/pnas.032426699 [PubMed: 11854509]
- Belden WJ, Larrondo LF, Froehlich AC, Shi M, Chen CH, Loros JJ, Dunlap JC. The band mutation in *Neurospora crassa* is a dominant allele of *ras-1* implicating RAS signaling in circadian output. *Genes Dev*. 2007; 21(12):1494–1505. 21/12/1494 [pii]. DOI: 10.1101/gad.1551707 [PubMed: 17575051]
- Bell-Pedersen D, Crosthwaite SK, Lakin-Thomas PL, Merrow M, Okland M. The *Neurospora* circadian clock: simple or complex? *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 2001; 356(1415):1697–1709. DOI: 10.1098/rstb.2001.0968 [PubMed: 11710976]
- Bertrand H, Chan BS, Griffiths AJ. Insertion of a foreign nucleotide sequence into mitochondrial DNA causes senescence in *Neurospora intermedia*. *Cell*. 1985; 41(3):877–884. [PubMed: 2408762]
- Bertrand H, Griffiths AJ, Court DA, Cheng CK. An extrachromosomal plasmid is the etiological precursor of *kal*DNA insertion sequences in the mitochondrial chromosome of senescent *neurospora*. *Cell*. 1986; 47(5):829–837. [PubMed: 2946419]
- Blakely RM, Srb AM. Studies of the genetics and physiology of a nitrate non-utilizing strain of *Neurospora*. *Neurospora Newsletter*. 1962:5–6.
- Bluhm BH, Burnham AM, Dunkle LD. A circadian rhythm regulating hyphal melanization in *Cercospora kikuchii*. *Mycologia*. 2010; 102(6):1221–1228. DOI: 10.3852/09-041 [PubMed: 20943572]
- Brandt WH. Zonation in a prolineless strain of *Neurospora*. *Mycologia*. 1953; 45(2):194–208.
- Brunner M, Schafmeier T. Transcriptional and post-transcriptional regulation of the circadian clock of cyanobacteria and *Neurospora*. *Genes Dev*. 2006; 20(9):1061–1074. [PubMed: 16651653]
- Campbell JL, Turner BC. Recombination block in the Spore killer region of *Neurospora*. *Genome*. 1987; 29(1):129–135. [PubMed: 3582973]
- Catcheside DE. Genes in *Neurospora* that suppress recombination when they are heterozygous. *Genetics*. 1981; 98(1):55–76. [PubMed: 6461572]
- Catcheside DG. Occurrence in wild strains of *Neurospora crassa* of genes controlling genetic recombination. *Aust J Biol Sci*. 1975; 28(2):213–225. [PubMed: 126680]
- Chang B, Nakashima H. Effects of light-dark cycles on the circadian conidiation rhythm in *Neurospora crassa*. *Journal of Plant Research*. 1997; 110(4):449–453.
- Chen CH, DeMay BS, Gladfelter AS, Dunlap JC, Loros JJ. Physical interaction between VIVID and white collar complex regulates photoadaptation in *Neurospora*. *Proc Natl Acad Sci U S A*. 2010; 107(38):16715–16720. 1011190107 [pii]. DOI: 10.1073/pnas.1011190107 [PubMed: 20733070]
- Cheng P, He Q, He Q, Wang L, Liu Y. Regulation of the *Neurospora* circadian clock by an RNA helicase. *Genes Dev*. 2005; 19(2):234–241. [PubMed: 15625191]
- Cheng P, He Q, He Q, Wang L, Liu Y. Regulation of the *Neurospora* circadian clock by an RNA helicase. *Genes Dev*. 2005; 19(2):234–241. DOI: 10.1101/gad.1266805 [PubMed: 15625191]
- Cheng P, He Q, Yang Y, Wang L, Liu Y. Functional conservation of light, oxygen, or voltage domains in light sensing. *Proc Natl Acad Sci U S A*. 2003; 100(10):5938–5943. [PubMed: 12719523]

- Cheng P, Yang Y, Gardner KH, Liu Y. PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. *Mol and Cell Biol*. 2002; 22(2):517–524. [PubMed: 11756547]
- Cheng P, Yang Y, Gardner KH, Liu Y. PAS domain-mediated WC-1/WC-2 interaction is essential for maintaining the steady-state level of WC-1 and the function of both proteins in circadian clock and light responses of *Neurospora*. *Mol Cell Biol*. 2002; 22(2):517–524. [PubMed: 11756547]
- Cheng P, Yang Y, Liu Y. Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proc Natl Acad Sci U S A*. 2001; 98(13):7408–7413. 98/13/7408 [pii]. DOI: 10.1073/pnas.121170298 [PubMed: 11416214]
- Cheng P, Yang Y, Wang L, He Q, Liu Y. WHITE COLLAR-1, a multifunctional *Neurospora* protein involved in the circadian feedback loops, light sensing, and transcription repression of *wc-2*. *J Biol Chem*. 2003; 278(6):3801–3808. [PubMed: 12454012]
- Christensen MK, Falkeid G, Loros JJ, Dunlap JC, Lillo C, Ruoff P. A nitrate-induced *frq*-less oscillator in *Neurospora crassa*. *J Biol Rhythms*. 2004; 19(4):280–286. DOI: 10.1177/0748730404265532 [PubMed: 15245647]
- Collins RA, Lambowitz AM. Structural variations and optional introns in the mitochondrial DNAs of *Neurospora* strains isolated from nature. *Plasmid*. 1983; 9(1):53–70. [PubMed: 6300945]
- Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, Litvinkova L, ... Dunlap JC. A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci U S A*. 2006; 103(27):10352–10357. 0601456103 [pii]. DOI: 10.1073/pnas.0601456103 [PubMed: 16801547]
- Coluccio MP, Sanchez SE, Kasulin L, Yanovsky MJ, Botto JF. Genetic mapping of natural variation in a shade avoidance response: ELF3 is the candidate gene for a QTL in hypocotyl growth regulation. *J Exp Bot*. 2011; 62(1):167–176. DOI: 10.1093/jxb/erq253 [PubMed: 20713464]
- Conrad KS, Hurley JM, Widom J, Ringelberg CS, Loros JJ, Dunlap JC, Crane BR. Structure of the frequency-interacting RNA helicase: a protein interaction hub for the circadian clock. *EMBO J*. 2016; 35(15):1707–1719. DOI: 10.15252/embj.201694327 [PubMed: 27340124]
- Correa A, Lewis ZA, Greene AV, March IJ, Gomer RH, Bell-Pedersen D. Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proc Natl Acad Sci U S A*. 2003; 100(23):13597–13602. DOI: 10.1073/pnas.2233734100 [PubMed: 14597725]
- Dawson A, King VM, Bentley GE, Ball GF. Photoperiodic control of seasonality in birds. *J Biol Rhythms*. 2001; 16(4):365–380. DOI: 10.1177/074873001129002079 [PubMed: 11506381]
- Dettman JR, Anderson JB, Kohn LM. Divergent adaptation promotes reproductive isolation among experimental populations of the filamentous fungus *Neurospora*. *BMC Evol Biol*. 2008; 8:35.doi: 10.1186/1471-2148-8-35 [PubMed: 18237415]
- Dettman JR, Anderson JB, Kohn LM. Genome-wide investigation of reproductive isolation in experimental lineages and natural species of *Neurospora*: identifying candidate regions by microarray-based genotyping and mapping. *Evolution*. 2010; 64(3):694–709. Epub 2009 Oct 5.<p>. DOI: 10.1111/j.1558-5646.2009.00863.x [PubMed: 19817850]
- Dettman JR, Harbinski FM, Taylor JW. Ascospore morphology is a poor predictor of the phylogenetic relationships of *Neurospora* and *Gelasinospora*. *Fungal Genet Biol*. 2001; 34(1):49–61. [PubMed: 11567551]
- Dettman JR, Jacobson DJ, Taylor JW. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. *Evolution Int J Org Evolution*. 2003; 57(12): 2703–2720.
- Dettman JR, Jacobson DJ, Taylor JW. Multilocus sequence data reveal extensive phylogenetic species diversity within the *Neurospora discreta* complex. *Mycologia*. 2006; 98(3):436–446. [PubMed: 17040072]
- Dettman JR, Jacobson DJ, Turner E, Pringle A, Taylor JW. Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. *Evolution Int J Org Evolution*. 2003; 57(12):2721–2741.
- Dodge BO. A new dominant lethal in *Neurospora*. *Journal of Heredity*. 1939; 30:467–474.
- Dunlap JC, Loros JJ. Making Time: Conservation of Biological Clocks from Fungi to Animals. *Microbiol Spectr*. 2017; 5(3)doi: 10.1128/microbiolspec.FUNK-0039-2016

- Eberhart BM. Exogenous enzymes of *Neurospora* conidia and mycelia. *J Cell Comp Physiol.* 1961; 58:11–16. [PubMed: 13725544]
- Eberhart BM, Beck RS, Goolsby KM. Cellulase of *Neurospora crassa*. *J Bacteriol.* 1977; 130(1):181–186. [PubMed: 15975]
- Edwards KD, Lynn JR, Gyula P, Nagy F, Millar AJ. Natural allelic variation in the temperature-compensation mechanisms of the *Arabidopsis thaliana* circadian clock. *Genetics.* 2005; 170(1): 387–400. DOI: 10.1534/genetics.104.035238 [PubMed: 15781708]
- Ellison CE, Hall C, Kowbel D, Welch J, Brem RB, Glass NL, Taylor JW. Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proc Natl Acad Sci U S A.* 2011; 108(7):2831–2836. DOI: 10.1073/pnas.1014971108 [PubMed: 21282627]
- Elvin M, Loros JJ, Dunlap JC, Heintzen C. The PAS/LOV protein VIVID supports a rapidly dampened daytime oscillator that facilitates entrainment of the *Neurospora* circadian clock. *Genes Dev.* 2005; 19(21):2593–2605. [PubMed: 16264193]
- Emerson MR. Chemical activation of ascospore germination in *Neurospora crassa*. *J Bacteriol.* 1948; 55(3):327–330. [PubMed: 16561462]
- Forni D, Pozzoli U, Cagliani R, Tresoldi C, Menozzi G, Riva S, ... Sironi M. Genetic adaptation of the human circadian clock to day-length latitudinal variations and relevance for affective disorders. *Genome Biol.* 2014; 15(10):499.doi: 10.1186/s13059-014-0499-7 [PubMed: 25358694]
- Frederick L, Uecker FA, Benjamin CR. A new species of *Neurospora* from soil of West Pakistan. *Mycologia.* 1969; 61(6):1077–1084. [PubMed: 5379283]
- Friborg O, Bjorvatn B, Amponsah B, Pallesen S. Associations between seasonal variations in day length (photoperiod), sleep timing, sleep quality and mood: a comparison between Ghana (5 degrees) and Norway (69 degrees). *J Sleep Res.* 2012; 21(2):176–184. DOI: 10.1111/j.1365-2869.2011.00982.x [PubMed: 22074234]
- Froehlich AC, Liu Y, Loros JJ, Dunlap JC. White Collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science.* 2002; 297:815–819. [PubMed: 12098706]
- Froehlich AC, Noh B, Vierstra RD, Loros J, Dunlap JC. Genetic and molecular analysis of phytochromes from the filamentous fungus *Neurospora crassa*. *Eukaryot Cell.* 2005; 4(12):2140–2152. [PubMed: 16339731]
- Garceau NY, Liu Y, Loros JJ, Dunlap JC. Alternative initiation of translation and time-specific phosphorylation yield multiple forms of the essential clock protein FREQUENCY. *Cell.* 1997; 89:469–476. [PubMed: 9150146]
- Gaston KJ. Global patterns in biodiversity. *Nature.* 2000; 405(6783):220–227. DOI: 10.1038/35012228 [PubMed: 10821282]
- Gill S, Panda S. Feeding mistiming decreases reproductive fitness in flies. *Cell Metab.* 2011; 13(6): 613–614. DOI: 10.1016/j.cmet.2011.05.003 [PubMed: 21641540]
- Glossop NRJ, Lyons LC, Hardin PE. Interlocked feedback loops within the *Drosophila* circadian oscillator. *Science.* 1999; 286:766–768. [PubMed: 10531060]
- Goddard DR. The Reversible Heat Activation Inducing Germination and Increased Respiration in the Ascospores of *Neurospora Tetrasperma*. *J Gen Physiol.* 1935; 19(1):45–60. [PubMed: 19872913]
- Goodrich-Tanrikulu M, Stafford AE, Jacobson DJ. A systematic survey of the fatty acid composition of *Neurospora* strains. *Fungal Genetics Reports.* 1999;8.
- Görl M, Merrow M, Huttner B, Johnson J, Roenneberg T, Brunner M. A PEST-like element in FREQUENCY determines the length of the circadian period in *Neurospora crassa*. *EMBO J.* 2001; 20(24):7074–7084. [PubMed: 11742984]
- Granshaw T, Tsukamoto M, Brody S. Circadian rhythms in *Neurospora crassa*: farnesol or geraniol allow expression of rhythmicity in the otherwise arrhythmic strains *frq10*, *wc-1*, and *wc-2*. *J Biol Rhythms.* 2003; 18(4):287–296. DOI: 10.1177/0748730403255934 [PubMed: 12932081]
- Green RM, Tingay S, Wang ZY, Tobin EM. Circadian rhythms confer a higher level of fitness to *Arabidopsis* plants. *Plant Physiol.* 2002; 129(2):576–584. DOI: 10.1104/pp.004374 [PubMed: 12068102]
- Greene AV, Keller N, Haas H, Bell-Pedersen D. A circadian oscillator in *Aspergillus* spp. regulates daily development and gene expression. *Eukaryot Cell.* 2003; 2(2):231–237. [PubMed: 12684372]

- Griffiths AJ. Fungal senescence. *Annu Rev Genet.* 1992; 26:351–372. DOI: 10.1146/annurev.ge.26.120192.002031 [PubMed: 1482117]
- Griffiths AJ. Natural plasmids of filamentous fungi. *Microbiol Rev.* 1995; 59(4):673–685. [PubMed: 8531891]
- Griffiths AJ, Bertrand H. Unstable cytoplasm in Hawaiian strains of *Neurospora intermedia*. *Curr Genet.* 1984; 8(5):387–398. DOI: 10.1007/BF00419828 [PubMed: 24177819]
- Grindle M, Davis RH. Variations in enzyme phenotypes and their underlying genotypes among wild strains of the genus *Neurospora*. *Genet Res.* 1970; 15(1):99–107. [PubMed: 5415230]
- Grove G, Marzluf GA. Identification of the product of the major regulatory gene of the nitrogen control circuit of *Neurospora crassa* as a nuclear DNA-binding protein. *J Biol Chem.* 1981; 256(1):463–470. [PubMed: 6450203]
- Guo J, Cheng P, Liu Y. Functional significance of FRH in regulating the phosphorylation and stability of *Neurospora* circadian clock protein FRQ. *J Biol Chem.* 2010; 285(15):11508–11515. DOI: 10.1074/jbc.M109.071688 [PubMed: 20159972]
- Hanson MA, Marzluf GA. Control of the synthesis of a single enzyme by multiple regulatory circuits in *Neurospora crassa*. *Proc Natl Acad Sci U S A.* 1975; 72(4):1240–1244. [PubMed: 124058]
- Hau M. Timing of breeding in variable environments: tropical birds as model systems. *Horm Behav.* 2001; 40(2):281–290. DOI: 10.1006/hbeh.2001.1673 [PubMed: 11534993]
- He C, Nastasja de G, Bok JW, Griffiths AJ. Kalilo plasmids are a family of four distinct members with individual global distributions across species. *Curr Genet.* 2000; 37(1):39–44. [PubMed: 10672443]
- He Q, Cha J, He Q, Lee HC, Yang Y, Liu Y. CKI and CKII mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the *Neurospora* circadian negative feedback loop. *Genes Dev.* 2006; 20(18):2552–2565. DOI: 10.1101/gad.1463506 [PubMed: 16980584]
- He Q, Cha J, Lee HC, Yang Y, Liu Y. CKI and CKII mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the *Neurospora* circadian negative feedback loop. *Genes Dev.* 2006; 20(18):2552–2565. [PubMed: 16980584]
- He Q, Cheng P, Yang Y, Wang L, Gardner KH, Liu Y. White Collar-1, a DNA binding transcription factor and a light sensor. *Science.* 2002; 297:840–843. [PubMed: 12098705]
- He Q, Cheng P, Yang Y, Yu H, Liu Y. FWD1-mediated degradation of FREQUENCY in *Neurospora* establishes a conserved mechanism for circadian clock regulation. *EMBO J.* 2003; 22(17):4421–4430. [PubMed: 12941694]
- He Q, Liu Y. Degradation of the *Neurospora* circadian clock protein FREQUENCY through the ubiquitin-proteasome pathway. *Biochem Soc Trans.* 2005a; 33(Pt 5):953–956. [PubMed: 16246019]
- He Q, Liu Y. Molecular mechanism of light responses in *Neurospora*: from light-induced transcription to photoadaptation. *Genes Dev.* 2005b; 19(23):2888–2899. [PubMed: 16287715]
- Heintzen C, Liu Y. The *Neurospora crassa* circadian clock. *Adv Genet.* 2007; 58:25–66. S0065-2660(06)58002-2 [pii]. DOI: 10.1016/S0065-2660(06)58002-2 [PubMed: 17452245]
- Heintzen C, Loros JJ, Dunlap JC. The PAS protein VIVID defines a clock-associated feedback loop that represses light input, modulates gating, and regulates clock resetting. *Cell.* 2001; 104:453–464. [PubMed: 11239402]
- Hevia MA, Canessa P, Larrondo LF. Circadian clocks and the regulation of virulence in fungi: Getting up to speed. *Semin Cell Dev Biol.* 2016; 57:147–155. DOI: 10.1016/j.semcdb.2016.03.021 [PubMed: 27039027]
- Hevia MA, Canessa P, Muller-Esparza H, Larrondo LF. A circadian oscillator in the fungus *Botrytis cinerea* regulates virulence when infecting *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A.* 2015; 112(28):8744–8749. DOI: 10.1073/pnas.1508432112 [PubMed: 26124115]
- Horowitz NH, Fling M, Macleod H, Sueoka N. A genetic study of two new structural forms of tyrosinase in *Neurospora*. *Genetics.* 1961; 46:1015–1024. [PubMed: 13715943]
- Hunt SM, Thompson S, Elvin M, Heintzen C. VIVID interacts with the WHITE COLLAR complex and FREQUENCY-interacting RNA helicase to alter light and clock responses in *Neurospora*. *Proc*

- Natl Acad Sci U S A. 2010; 107(38):16709–16714. 1009474107 [pii]. DOI: 10.1073/pnas.1009474107 [PubMed: 20807745]
- Hurley J, Loros JJ, Dunlap JC. Dissecting the mechanisms of the clock in *Neurospora*. *Methods Enzymol.* 2015; 551:29–52. DOI: 10.1016/bs.mie.2014.10.009 [PubMed: 25662450]
- Hurley JM, Larrondo LF, Loros JJ, Dunlap JC. Conserved RNA helicase FRH acts nonenzymatically to support the intrinsically disordered *Neurospora* clock protein FRQ. *Mol Cell.* 2013; 52(6):832–843. DOI: 10.1016/j.molcel.2013.11.005 [PubMed: 24316221]
- Jacobson DJ, Dettman JR, Adams RI, Boesl C, Sultana S, Roenneberg T, ... Taylor JW. New findings of *Neurospora* in Europe and comparisons of diversity in temperate climates on continental scales. *Mycologia.* 2006; 98(4):550–559. [PubMed: 17139848]
- Jacobson DJ, Powell AJ, Dettman JR, Saenz GS, Barton MM, Hiltz MD, ... Natvig DO. *Neurospora* in temperate forests of western North America. *Mycologia.* 2004; 96(1):66–74. [PubMed: 21148830]
- Kaldi K, Gonzalez BH, Brunner M. Transcriptional regulation of the *Neurospora* circadian clock gene *wc-1* affects the phase of circadian output. *EMBO Rep.* 2006; 7(2):199–204. [PubMed: 16374510]
- Kim TS, Booth JG, Gauch HG Jr, Sun Q, Park J, Lee YH, Lee K. Simple sequence repeats in *Neurospora crassa*: distribution, polymorphism and evolutionary inference. *BMC Genomics.* 2008; 9:31.doi: 10.1186/1471-2164-9-31 [PubMed: 18215294]
- Kim TS, Logsdon BA, Park S, Mezey JG, Lee K. Quantitative trait loci for the circadian clock in *Neurospora crassa*. *Genetics.* 2007; 177(4):2335–2347. DOI: 10.1534/genetics.107.077958 [PubMed: 17947430]
- Lakin-Thomas PL. Choline depletion, *frq* mutations, and temperature compensation of the circadian rhythm in *Neurospora crassa*. *J Biol Rhythms.* 1998; 13(4):268–277. DOI: 10.1177/074873098129000101 [PubMed: 9711502]
- Lakin-Thomas PL, Bell-Pedersen D, Brody S. The genetics of circadian rhythms in *Neurospora*. *Adv Genet.* 2011; 74:55–103. DOI: 10.1016/B978-0-12-387690-4.00003-9 [PubMed: 21924975]
- Lakin-Thomas PL, Brody S. Circadian rhythms in *Neurospora crassa*: lipid deficiencies restore robust rhythmicity to null frequency and white-collar mutants. *Proceedings of the National Academy of Sciences of the United States of America.* 2000; 97(1):256–261. [PubMed: 10618405]
- Lankinen P, Forsman P. Independence of genetic geographical variation between photoperiodic diapause, circadian eclosion rhythm, and Thr-Gly repeat region of the period gene in *Drosophila littoralis*. *J Biol Rhythms.* 2006; 21(1):3–12. DOI: 10.1177/0748730405283418 [PubMed: 16461980]
- Lauinger L, Diernfellner A, Falk S, Brunner M. The RNA helicase FRH is an ATP-dependent regulator of CK1a in the circadian clock of *Neurospora crassa*. *Nat Commun.* 2014; 5:3598.doi: 10.1038/ncomms4598 [PubMed: 24710172]
- Lee K. Asexual and sexual developments of *Neurospora crassa* on natural substrata. *Fungal Ecology.* 2012; 5(2):223–229. doi:<http://dx.doi.org/10.1016/j.funeco.2011.09.001>.
- Lee K, Dighton J. *Neurospora*, a potential fungal organism for experimental and evolutionary ecology. *Fungal Biology Reviews.* 2010; 24(3–4):85–89. doi:<http://dx.doi.org/10.1016/j.fbr.2010.09.001>.
- Lee, K., Dighton, J. Advancement of Functional Genomics of a Model Species of *Neurospora* and Its Use for Ecological Genomics of Soil Fungi. In: Horwitz, BA.Mukherjee, PK.Mukherjee, M., Kubicek, CP., editors. *Genomics of Soil- and Plant-Associated Fungi.* Vol. 36. Springer; Berlin Heidelberg: 2013. p. 29-44.
- Lee K, Dunlap CJ, Loros JJ. Roles for WHITE COLLAR-1 in circadian and general photoperception in *Neurospora crassa*. *Genetics.* 2003; 163(1):103–114. [PubMed: 12586700]
- Lee K, Loros JJ, Dunlap JC. Interconnected feedback loops in the *Neurospora* circadian system. *Science.* 2000; 289:107–110. [PubMed: 10884222]
- Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D. Diversity of flowering responses in wild *Arabidopsis thaliana* strains. *PLoS Genet.* 2005; 1(1):109–118. DOI: 10.1371/journal.pgen.0010006 [PubMed: 16103920]
- Leslie JF, Raju NB. Recessive mutations from natural populations of *Neurospora crassa* that are expressed in the sexual diplophase. *Genetics.* 1985; 111(4):759–777. [PubMed: 2933298]
- Levine WB, Marzluf GA. Isolation and characterization of cadmium-resistant mutants of *Neurospora crassa*. *Can J Microbiol.* 1989; 35(3):359–365. [PubMed: 2525066]

- Lewis MT, Feldman JF. Evolution of the frequency (frq) clock locus in Ascomycete fungi. *Mol Biol Evol.* 1996; 13(9):1233–1241. [PubMed: 8896376]
- Li N, Joska TM, Ruesch CE, Coster SJ, Belden WJ. The frequency natural antisense transcript first promotes, then represses, frequency gene expression via facultative heterochromatin. *Proc Natl Acad Sci U S A.* 2015; 112(14):4357–4362. DOI: 10.1073/pnas.1406130112 [PubMed: 25831497]
- Liu Y, Loros J, Dunlap JC. Phosphorylation of the *Neurospora* clock protein FREQUENCY determines its degradation rate and strongly influences the period length of the circadian clock. *Proc Natl Acad Sci U S A.* 2000; 97(1):234–239. [PubMed: 10618401]
- Loros JJ, Richman A, Feldman JF. A recessive circadian clock mutation at the frq locus of *Neurospora crassa*. *Genetics.* 1986; 114(4):1095–1110. [PubMed: 2948874]
- Low KH, Chen WF, Yildirim E, Edery I. Natural variation in the *Drosophila melanogaster* clock gene period modulates splicing of its 3'-terminal intron and mid-day siesta. *PLoS ONE.* 2012; 7(11):e49536.doi: 10.1371/journal.pone.0049536 [PubMed: 23152918]
- Low KH, Lim C, Ko HW, Edery I. Natural variation in the splice site strength of a clock gene and species-specific thermal adaptation. *Neuron.* 2008; 60(6):1054–1067. DOI: 10.1016/j.neuron.2008.10.048 [PubMed: 19109911]
- Mahadevan PR, Eberhart B. Aryl Beta-Glucosidase of Some *Neurospora* Strains. *Biochim Biophys Acta.* 1964; 90:214–215. [PubMed: 14201170]
- Maheshwari R, Navaraj A. Senescence in fungi: the view from *Neurospora*. *FEMS microbiology letters.* 2008; 280(2):135–143. DOI: 10.1111/j.1574-6968.2007.01027.x [PubMed: 18093134]
- Mannella CA, Pittenger TH, Lambowitz AM. Transmission of mitochondrial deoxyribonucleic acid in *Neurospora crassa* sexual crosses. *J Bacteriol.* 1979; 137(3):1449–1451. [PubMed: 155689]
- McCluskey K, Wiest A, Plamann M. The Fungal Genetics Stock Center: a repository for 50 years of fungal genetics research. *J Biosci.* 2010; 35(1):119–126. [PubMed: 20413916]
- Mehra A, Shi M, Baker CL, Colot HV, Loros JJ, Dunlap JC. A role for casein kinase 2 in the mechanism underlying circadian temperature compensation. *Cell.* 2009; 137(4):749–760. DOI: 10.1016/j.cell.2009.03.019 [PubMed: 19450520]
- Merrow M, Brunner M, Roenneberg T. Assignment of circadian function for the *Neurospora* clock gene frequency. *Nature.* 1999; 399(6736):584–586. DOI: 10.1038/21190 [PubMed: 10376598]
- Merrow MW, Dunlap JC. Intergeneric complementation of a circadian rhythmicity defect: phylogenetic conservation of structure and function of the clock gene frequency. *EMBO J.* 1994; 13(10):2257–2266. [PubMed: 8194516]
- Merrow MW, Garceau NY, Dunlap JC. Dissection of a circadian oscillation into discrete domains. *Proc Natl Acad Sci U S A.* 1997; 94(8):3877–3882. [PubMed: 9108072]
- Metzenberg RL, Ahlgren SK. Structural and regulatory control of aryl sulfatase in *Neurospora*: the use of interspecific differences in structural genes. *Genetics.* 1971; 68(3):369–381. [PubMed: 4942555]
- Michael TP, Park S, Kim TS, Booth J, Byer A, Sun Q, ... Lee K. Simple Sequence Repeats Provide a Substrate for Phenotypic Variation in the *Neurospora crassa* Circadian Clock. *PLoS One.* 2007; 2(8):e795.doi: 10.1371/journal.pone.0000795 [PubMed: 17726525]
- Michael TP, Salome PA, Yu HJ, Spencer TR, Sharp EL, McPeck MA, ... McClung CR. Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science.* 2003; 302(5647):1049–1053. DOI: 10.1126/science.1082971 [PubMed: 14605371]
- Miller BH, Olson SL, Turek FW, Levine JE, Horton TH, Takahashi JS. Circadian clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr Biol.* 2004; 14(15):1367–1373. DOI: 10.1016/j.cub.2004.07.055 [PubMed: 15296754]
- Morgan LW, Feldman JF. Preliminary investigation of the circadian rhythms of wild-collected *Neurospora* strains. *Fungal Genetics Newsletter.* 1998:30–31.
- Mylyk OM. Heterokaryon incompatibility genes in *Neurospora crassa* detected using duplication-producing chromosome rearrangements. *Genetics.* 1975; 80(1):107–124. [PubMed: 124287]
- Mylyk OM. Heteromorphism for heterokaryon incompatibility genes in natural populations of *Neurospora Crassa*. *Genetics.* 1976; 83(2):275–284. [PubMed: 17248714]

- Natvig DO, May G, Taylor JW. Distribution and Evolutionary Significance of Mitochondrial Plasmids in *Neurospora* Spp. *Journal of Bacteriology*. 1984; 159(1):288–293. [PubMed: 6330036]
- Navaraj A, Pandit A, Maheshwari R. Senescent: a new *Neurospora crassa* nuclear gene mutant derived from nature exhibits mitochondrial abnormalities and a “death” phenotype. *Fungal Genet Biol*. 2000; 29(3):165–173. DOI: 10.1006/fgbi.2000.1193 [PubMed: 10882533]
- Newmeyer D. A suppressor of the heterokaryon-incompatibility associated with mating type in *Neurospora crassa*. *Can J Genet Cytol*. 1970; 12(4):914–926. [PubMed: 5512565]
- Nsa IY, Karunarathna N, Liu X, Huang H, Boettger B, Bell-Pedersen D. A novel cryptochrome-dependent oscillator in *Neurospora crassa*. *Genetics*. 2015; 199(1):233–245. DOI: 10.1534/genetics.114.169441 [PubMed: 25361899]
- Oliveira AG, Stevani CV, Waldenmaier HE, Viviani V, Emerson JM, Loros JJ, Dunlap JC. Circadian control sheds light on fungal bioluminescence. *Curr Biol*. 2015; 25(7):964–968. DOI: 10.1016/j.cub.2015.02.021 [PubMed: 25802150]
- Park S, Lee K. Inverted race tube assay for a circadian clock studies of the *Neurospora* Accessions. *Fungal Genetics Newsletter*. 2004:12–14.
- Perkins DD. The use of duplication-generating rearrangements for studying heterokaryon incompatibility genes in *Neurospora*. *Genetics*. 1975; 80(1):87–105. [PubMed: 124288]
- Perkins DD, Metzenberg RL, Raju NB, Selker EU, Barry EG. Reversal of a *Neurospora* translocation by crossing over involving displaced rDNA, and methylation of the rDNA segments that result from recombination. *Genetics*. 1986; 114(3):791–817. [PubMed: 2947829]
- Perkins DD, Radford A, Newmeyer D, Bjorkman M. Chromosomal loci of *Neurospora crassa*. *Microbiol Rev*. 1982; 46(4):426–570. [PubMed: 6219280]
- Perkins DD, Turner BC. *Neurospora* from natural populations: Toward the population biology of a haploid eukaryote. *Experimental Mycology*. 1988; 12:91–131.
- Perkins DD, Turner BC, Barry EG. Strains of *Neurospora* collected from nature. *Evolution*. 1976; 30:281–313. [PubMed: 28563048]
- Pittendrigh CS, Bruce VG, Rosensweig NS, Rubin ML. Growth Patterns in *Neurospora*: A Biological Clock in *Neurospora*. *Nature*. 1959; 184:169–170. DOI: 10.1038/184169a0
- Pittendrigh CS, Kyner WT, Takamura T. The amplitude of circadian oscillations: temperature dependence, latitudinal clines, and the photoperiodic time measurement. *J Biol Rhythms*. 1991; 6(4):299–313. DOI: 10.1177/074873049100600402 [PubMed: 1773097]
- Pittendrigh CS, Takamura T. Latitudinal clines in the properties of a circadian pacemaker. *J Biol Rhythms*. 1989; 4(2):217–235. [PubMed: 2519590]
- Pregueiro AM, Liu Q, Baker CL, Dunlap JC, Loros JJ. The *Neurospora* checkpoint kinase 2: a regulatory link between the circadian and cell cycles. *Science*. 2006; 313(5787):644–649. [PubMed: 16809488]
- Press MO, Lancot A, Queitsch C. PIF4 and ELF3 Act Independently in *Arabidopsis thaliana* Thermoresponsive Flowering. *PLoS ONE*. 2016; 11(8):e0161791.doi: 10.1371/journal.pone.0161791 [PubMed: 27564448]
- Pringle A, Taylor J. The fitness of filamentous fungi. *Trends Microbiol*. 2002; 10(10):474–481. [PubMed: 12377558]
- Querfurth C, Diernfellner AC, Gin E, Malzahn E, Hofer T, Brunner M. Circadian conformational change of the *Neurospora* clock protein FREQUENCY triggered by clustered hyperphosphorylation of a basic domain. *Mol Cell*. 2011; 43(5):713–722. DOI: 10.1016/j.molcel.2011.06.033 [PubMed: 21884974]
- Raju NB, Leslie JF. Cytology of recessive sexual-phase mutants from wild strains of *Neurospora crassa*. *Genome*. 1992; 35(5):815–826. [PubMed: 1427061]
- Raschke A, Ibanez C, Ullrich KK, Anwer MU, Becker S, Glockner A, ... Quint M. Natural variants of ELF3 affect thermomorphogenesis by transcriptionally modulating PIF4-dependent auxin response genes. *BMC Plant Biol*. 2015; 15:197.doi: 10.1186/s12870-015-0566-6 [PubMed: 26269119]
- Reddy MM, Threlkeld SF. Genetic studies of isozymes in *Neurospora*. I. A study of eight species. *Can J Genet Cytol*. 1971a; 13(2):298–305. [PubMed: 5110193]

- Reddy MM, Threlkeld SF. Isozyme variation in natural populations of *Neurospora*. 1971b Retrieved from.
- Reddy MM, Threlkeld SF. Genetic studies of isozymes in *Neurospora*. II. Esterases. *Can J Genet Cytol.* 1972; 14(3):527–531. [PubMed: 4653143]
- Reierth E, Van't Hof TJ, Stokkan KA. Seasonal and daily variations in plasma melatonin in the high-arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *J Biol Rhythms.* 1999; 14(4):314–319. DOI: 10.1177/074873099129000731 [PubMed: 10447312]
- Rieck A, Griffiths AJ, Bertrand H. Mitochondrial variants of *Neurospora intermedia* from nature. *Can J Genet Cytol.* 1982; 24(6):741–759. [PubMed: 6303535]
- Roenneberg T, Taylor W. Automated recordings of bioluminescence with special reference to the analysis of circadian rhythms. *Methods Enzymol.* 2000; 305:104–119. [PubMed: 10812594]
- Romero-Olivares AL, Taylor JW, Treseder KK. *Neurospora discreta* as a model to assess adaptation of soil fungi to warming. *BMC Evol Biol.* 2015; 15:198.doi: 10.1186/s12862-015-0482-2 [PubMed: 26377599]
- Rosato E, Kyriacou CP. The role of natural selection in circadian behaviour: a molecular-genetic approach. *Essays Biochem.* 2011; 49(1):71–85. DOI: 10.1042/BSE0490071 [PubMed: 21819385]
- Ruegg C, Ammer D, Lerch K. Comparison of Amino-Acid-Sequence and Thermostability of Tyrosinase from 3 Wild-Type Strains of *Neurospora crassa*. *Journal of Biological Chemistry.* 1982; 257(11):6420–6426. [PubMed: 6210697]
- Salichos L, Rokas A. The diversity and evolution of circadian clock proteins in fungi. *Mycologia.* 2010; 102(2):269–278. [PubMed: 20361495]
- Sargent ML, Kaltenborn SH. Effects of medium composition and carbon dioxide on circadian conidiation in *neurospora*. *Plant Physiol.* 1972; 50(1):171–175. [PubMed: 16658116]
- Sargent ML, Woodward DO. Genetic determinants of circadian rhythmicity in *Neurospora*. *J Bacteriol.* 1969; 97(2):861–866. [PubMed: 5773032]
- Sawyer LA, Hennessy JM, Peixoto AA, Rosato E, Parkinson H, Costa R, Kyriacou CP. Natural variation in a *Drosophila* clock gene and temperature compensation. *Science.* 1997; 278(5346): 2117–2120. [PubMed: 9405346]
- Schafmeier T, Haase A, Káldi K, Scholz J, Fuchs M, Brunner M. Transcriptional Feedback of *Neurospora* Circadian Clock Gene by Phosphorylation-Dependent Inactivation of Its Transcription Factor. *Cell.* 2005; 122(2):235–246. [PubMed: 16051148]
- Schneider K, Perrino S, Oelhafen K, Li S, Zatzepin A, Lakin-Thomas P, Brody S. Rhythmic conidiation in constant light in vivid mutants of *Neurospora crassa*. *Genetics.* 2009; 181(3):917–931. DOI: 10.1534/genetics.108.097808 [PubMed: 19139144]
- Schwerdtfeger C, Linden H. VIVID is a flavoprotein and serves as a fungal blue light photoreceptor for photoadaptation. *EMBO J.* 2003; 22(18):4846–4855. [PubMed: 12970196]
- Selker EU, Stevens JN. Signal for DNA methylation associated with tandem duplication in *Neurospora crassa*. *Mol Cell Biol.* 1987; 7(3):1032–1038. [PubMed: 2951588]
- Shear CL, Dodge BO. Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. *Journal of Agricultural Research.* 1927; 34:1019–1042.
- Shearman LP, Sriram S, Weaver DR, Maywood ES, Chaves Ie, Zheng B, ... Reppert SM. Interacting molecular loops in the mammalian circadian clock. *Science.* 2000; 288:1013–1019. [PubMed: 10807566]
- Shi M, Collett M, Loros JJ, Dunlap JC. FRQ-interacting RNA helicase mediates negative and positive feedback in the *Neurospora* circadian clock. *Genetics.* 2010; 184(2):351–361. DOI: 10.1534/genetics.109.111393 [PubMed: 19948888]
- Shimomura K, Low-Zeddies SS, King DP, Steeves TD, Whiteley A, Kushla J, ... Takahashi JS. Genome-wide epistatic interaction analysis reveals complex genetic determinants of circadian behavior in mice. *Genome Res.* 2001; 11(6):959–980. DOI: 10.1101/gr.171601 [PubMed: 11381025]
- Shrode LB, Lewis ZA, White LD, Bell-Pedersen D, Ebbole DJ. *vvd* is required for light adaptation of conidiation-specific genes of *Neurospora crassa*, but not circadian conidiation. *Fungal Genet*

- Biol. 2001; 32(3):169–181. S1087-1845(01)91264-2 [pii]. DOI: 10.1006/fgbi.2001.1264 [PubMed: 11343403]
- Skupski MP, Jackson DA, Natvig DO. Phylogenetic analysis of heterothallic *Neurospora* species. Fungal Genet Biol. 1997; 21(1):153–162.
- Smith DA. A mutant affecting meiosis in *Neurospora*. Genetics. 1975; 80(1):125–133. [PubMed: 1137966]
- Smith ML, Bruhn JN, Anderson JB. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature. 1992; 356(6368):428.
- Spieth PT. Population genetics of allozyme variation in *Neurospora intermedia*. Genetics. 1975; 80(4):785–805. [PubMed: 1193373]
- Springer ML. Genetic control of fungal differentiation: The three sporulation pathways of *Neurospora crassa*. Bioessays. 1993; 15:365–374. [PubMed: 8357339]
- Springer ML, Yanofsky C. A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. Genes Dev. 1989; 3:559–571. [PubMed: 2524423]
- Svetec N, Zhao L, Saelao P, Chiu JC, Begun DJ. Evidence that natural selection maintains genetic variation for sleep in *Drosophila melanogaster*. BMC Evol Biol. 2015; 15:41.doi: 10.1186/s12862-015-0316-2 [PubMed: 25887180]
- Swarup K, Alonso-Blanco C, Lynn JR, Michaels SD, Amasino RM, Koornneef M, Millar AJ. Natural allelic variation identifies new genes in the *Arabidopsis* circadian system. Plant J. 1999; 20(1):67–77. [PubMed: 10571866]
- Tai FL. Two new species of *Neurospora*. Mycologia. 1935; 27:328–330.
- Talora C, Franchi L, Linden H, Ballario P, Macino G. Role of a white collar-1-white collar-2 complex in blue-light signal transduction. EMBO J. 1999; 18(18):4961–4968. DOI: 10.1093/emboj/18.18.4961 [PubMed: 10487748]
- Taylor JW. Fungal Evolutionary Biology and Mitochondrial-DNA. Experimental Mycology. 1986; 10(4):259–269. DOI: 10.1016/0147-5975(86)90011-3
- Taylor, JW., Koupel, T., Whitmer, S. Distribution of mitochondrial DNA length mutations in *Neurospora crassa* isolates from Florida and the Ivory Coast (abstract). Paper presented at the Mycological Society of America Newsletter; 1987.
- Taylor JW, Smolich BD, May G. An evolutionary comparison of homologous mitochondrial plasmid DNAs from three *Neurospora* species. Mol Gen Genet. 1985; 201(2):161–167. [PubMed: 3003521]
- Taylor JW, Smolich BD, May G. Evolution and Mitochondrial-DNA in *Neurospora-Crassa*. Evolution. 1986; 40(4):716–739. DOI: 10.2307/2408458 [PubMed: 28556147]
- Thom C, Ayers SH. Effect of pasteurization on mold spores. Journal of Agricultural Research. 1916; 6:153–166.
- Toyota K, Onai K, Nakashima H. A new *wc-1* mutant of *Neurospora crassa* shows unique light sensitivity in the circadian conidiation rhythm. Mol Gen Genomics. 2002; 268:56–61.
- Traeger S, Nowrousian M. Analysis of Circadian Rhythms in the Basal Filamentous Ascomycete *Pyronema confluens*. G3 (Bethesda). 2015; 5(10):2061–2071. DOI: 10.1534/g3.115.020461 [PubMed: 26254031]
- Turner BC, Perkins DD, Fairfield A. *Neurospora* from natural populations: a global study. Fungal Genet Biol. 2001; 32(2):67–92. DOI: 10.1006/fgbi.2001.1247 [PubMed: 11352529]
- Tuveson RW, Peterson JF. Virus-like particles in certain slow-growing strains of *Neurospora crassa*. Virology. 1972; 47(2):527–531. [PubMed: 4110285]
- Wang B, Kettenbach AN, Gerber SA, Loros JJ, Dunlap JC. *Neurospora WC-1* recruits SWI/SNF to remodel frequency and initiate a circadian cycle. PLoS Genet. 2014; 10(9):e1004599.doi: 10.1371/journal.pgen.1004599 [PubMed: 25254987]
- Weiss B, Turian G. A study of conidiation in *Neurospora crassa*. J Gen Microbiol. 1966; 44(3):407–418. DOI: 10.1099/00221287-44-3-407 [PubMed: 5971388]
- Westergaard M, Mitchell HK. *Neurospora V*. A synthetic medium favoring sexual reproduction. American Journal of Botany. 1947:573–577.

- Xu K, DiAngelo JR, Hughes ME, Hogenesch JB, Sehgal A. The circadian clock interacts with metabolic physiology to influence reproductive fitness. *Cell Metab.* 2011; 13(6):639–654. DOI: 10.1016/j.cmet.2011.05.001 [PubMed: 21641546]
- Xue Z, Ye Q, Anson SR, Yang J, Xiao G, Kowbel D, ... Liu Y. Transcriptional interference by antisense RNA is required for circadian clock function. *Nature.* 2014; 514(7524):650–653. DOI: 10.1038/nature13671 [PubMed: 25132551]
- Yang Y, Cheng P, Liu Y. Regulation of the *Neurospora* circadian clock by casein kinase II. *Genes Dev.* 2002; 16(8):994–1006. [PubMed: 11959847]
- Yang Y, Cheng P, Zhi G, Liu Y. Identification of a calcium/calmodulin-dependent protein kinase that phosphorylates the *Neurospora* circadian clock protein FREQUENCY. *J Biol Chem.* 2001; 276(44):41064–41072. [PubMed: 11551951]
- Yang Y, He Q, Cheng P, Wragg P, Yarden O, Liu Y. Distinct roles for PP1 and PP2A in the *Neurospora* circadian clock. *Genes Dev.* 2004; 18(3):255–260. [PubMed: 14871927]
- Young JL, Marzluf GA. Molecular comparison of the negative-acting nitrogen control gene, *nmr*, in *Neurospora crassa* and other *Neurospora* and fungal species. *Biochem Genet.* 1991; 29(9–10): 447–459. [PubMed: 1663340]
- Yu SA, Garrett K, Sussman AS. Genetic control of multiple forms of trehalase in *Neurospora crassa*. *Genetics.* 1971; 68(4):473–481. [PubMed: 5120655]
- Zalokar M. Studies on biosynthesis of carotenoids in *Neurospora crassa*. *Arch Biochem Biophys.* 1954; 50(1):71–80. [PubMed: 13159302]
- Zalokar M. Enzyme activity and cell differentiation in *Neurospora*. *American Journal of Botany.* 1959; 46:555–559.
- Zalokar M, Cochrane VW. Diphosphopyridine nucleotidase in the life cycle of *Neurospora crassa*. *American Journal of Botany.* 1956; 43(2):107–110.
- Zoltowski BD, Crane BR. Light activation of the LOV protein *vivid* generates a rapidly exchanging dimer. *Biochemistry.* 2008; 47(27):7012–7019. DOI: 10.1021/bi8007017 [PubMed: 18553928]

Figure 1.

Distribution of natural ecotypes of *N. crassa* (A) and *N. discreta* (B). Among the natural ecotypes deposited in Fungal Genetics Stock Center, we chose 114 *N. crassa* and 85 *N. discreta* strains that have a complete collection record without redundancy. The distributions of these representative strains are plotted by the collection sites (C). Error bars represent one standard deviation of the mean. The distribution between two species in the Northern hemisphere had a statistically significant difference (*t*-test, $p < 0.0001$).

Figure 2.

Overview of natural variation studies in fungal circadian clocks. Each natural strain has been successfully adapted to the local environment. Many layers of ambient environmental factors will function as selection forces. Thus, the variation observed in the circadian rhythm reflects the natural history of the organism. Among the circadian controlled phenotypes, asexual and sexual development in *Neurospora* have been extensively studied. Cellular oscillators: blue lines for expressed gene products, red lines for up-regulation (arrow) or down-regulation (line) of a gene, dotted line for unknown relationships. Images for a conidial development are modified from (Lee & Dighton, 2010) and from (Lee, 2012). Images of sexual development are modified from (Lee, 2012).

Table 1Natural variant studies in *Neurospora*.

Species	Category	Traits	Reference
	Chromosomal modifications	Satellite Tandom duplications Translocations	(Perkins, Metzenberg, Raju, Selker, & Barry, 1986) (Selker & Stevens, 1987) (Perkins & Turner, 1988)
	Enzymes	b-Glucosidase Cellulase Fatty acid synthesis Invertase Leucyl-tRNA synthetase Nitrogen regulation Ornithine transcarbamylase Protease Tyrosinase	(Mahadevan & Eberhart, 1964) (Eberhart, Beck, & Goolsby, 1977) (Goodrich-Tanrikulu, Stafford, & Jacobson, 1999) (Yu et al., 1971) (Beauchamp et al., 1977) (Young & Marzluf, 1991) (Grindle & Davis, 1970) (Hanson & Marzluf, 1975) (Horowitz et al., 1961; Ruegg et al., 1982)
	Cell cycle	Meiosis impaired Recombination repression Sexual phase recessives Synaptic sequence	(D. A. Smith, 1975) (D. G. Catcheside, 1975) (Leslie & Raju, 1985; Raju & Leslie, 1992) (D. E. Catcheside, 1981)
	Mitochondria	Mitochondrial DNA variants Mitochondrial plasmids Senescence	(Collins & Lambowitz, 1983; Mannella et al., 1979; Taylor, Smolich, & May, 1986) (Griffiths, 1995; Taylor, Smolich, & May, 1985 1985) (Navaraj, Pandit, & Maheshwari, 2000)
	Nutrients		(Leslie & Raju, 1985; Perkins et al., 1976)
	Reproductive Success		(J. R. Dettman, Jacobson, Turner, et al., 2003)
	Resistance to <i>Sk</i>		(Campbell & Turner, 1987)
	Resistance to toxic agents	Canavanine Cadmium resistance Surfactant resistant Triphenyl tetrazolium chloride	(Perkins, Radford, Newmeyer, & Bjorkman, 1982) (Levine & Marzluf, 1989) (Al-Saqr & Smith, 1980) (Perkins et al., 1982)
	Vegetative incompatibility	<i>het</i> loci Suppressor	(Mylyk, 1975, 1976; Perkins, 1975) (Newmeyer, 1970)
	Enzymes Mitochondria Speciation	Fatty acid synthesis Mitochondrial Plasmids Phylogenetic variation	(Goodrich-Tanrikulu et al., 1999) (C. He, Nastasja de, Bok, & Griffiths, 2000) (Jeremy R. Dettman et al., 2006)
<i>N. intermedia</i>	Chromosomal modifications	Tandom duplication Translocations	(Selker & Stevens, 1987) (Perkins & Turner, 1988)

Species	Category	Traits	Reference
	Enzymes	Allozymes	(Spieth, 1975)
		b-Glucosidases	(Perkins & Turner, 1988)
		Cellulase	(Perkins & Turner, 1988)
		Estreases	(Reddy & Threlkeld, 1972)
		Nitrate reductase	(Blakely & Srb, 1962)
		Tyrosinase	(Perkins & Turner, 1988)
		Microcycle conidiation	(Turner et al., 2001)
		Nitrogen regulation	(Young & Marzluf, 1991)
		Fatty acid synthesis	(Goodrich-Tanrikulu et al., 1999)
	Mitochondria	Mitochondrial DNA variants	(Collins & Lambowitz, 1983)
		Mitochondrial Plasmids	(Griffiths, 1995; Natvig, May, & Taylor, 1984)
		Senescence	(Bertrand, Chan, & Griffiths, 1985; Bertrand, Griffiths, Court, & Cheng, 1986; Griffiths & Bertrand, 1984; Rieck, Griffiths, & Bertrand, 1982)
	Resistance to toxic agents	Surfactant resistant	(Al-Saqr & Smith, 1980)
	Reproductive Success		(J. R. Dettman, Jacobson, Turner, et al., 2003)
	Spore killer	Resistance to <i>Sk</i>	(Campbell & Turner, 1987)
<i>N. sitophila</i>	Virus-like particles		(Perkins & Turner, 1988; Tuveson & Peterson, 1972)
	Chromosomal modifications	Tandom duplication	(Selker & Stevens, 1987)
	Enzymes	Amylases	(Reddy & Threlkeld, 1971a, 1971b, 1972)
		Nitrogen regulation	(Grove & Marzluf, 1981; Perkins & Turner, 1988; Young & Marzluf, 1991)
		Fatty acid synthesis	(Goodrich-Tanrikulu et al., 1999)
		Tyrosinase	(Perkins & Turner, 1988)
<i>N. tetrasperma</i>	Mitochondria	Mitochondrial Plasmids	(Griffiths, 1995)
		Mitochondrial DNA variants	(Collins & Lambowitz, 1983)
	Chromosomal modifications	Tandom duplication	(Selker & Stevens, 1987)
	Enzymes	Aryl-sulfatase	(Metzenberg & Ahlgren, 1971)
		Estreases	(Reddy & Threlkeld, 1971a)
		Fatty acid synthesis	(Goodrich-Tanrikulu et al., 1999)
	Mitochondria	Mitochondrial Plasmids	(Griffiths, 1995; Natvig et al., 1984)