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Effect of different light regimes on esterase isozyme profiles of three species of *Drosophila*

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Circadian rhythm has been identified in every organism studied, from unicellular marine algae to man, and in virtually all physiological and biochemical functions. The endogenous circadian system functions to organize behaviour and physiology to adapt to and anticipate environment changes in light and temperature. The present study is an attempt to understand enzyme profiles (alpha and beta esterases) of *Drosophila melanogaster* (Oregon-K strain), *Drosophila gangotrii* and *Drosophila jambulina* under light/dark (LD), continuous light (LL) and continuous dark (DD) conditions over 30 generations. A polyacrylamide gel electrophoresis (7.5% – native gel) was used to study the esterase isozyme banding patterns in three species of *Drosophila*. It has been noticed that there were three alpha esterase loci in *Drosophila* species which were designated as α-est 1, α-est 2 and α-est 3. Similarly there were three beta esterase loci which were designated as β-est 1, β-est 2 and β-est 3. Flies maintained in different light regimes showed differences in their allelic patterns with respect to alpha and beta esterases. It was observed that there was expression of some bands at a given light regime and the absence of the same in another regime. This shows that the different light regimes affect the expression of esterase isozymes.

Keywords: endogenous; physiology; esterase

Introduction

Circadian rhythms are daily cycles in the behaviour and physiology of an organism. The cycles are endogenously generated self-sustaining rhythms but can be influenced by environmental stimuli such as light and feeding (Schibler 2005). Circadian clocks in flies help to coordinate rhythmic feeding behaviour and regulate proper energy consumption and metabolism. Food consumption in *Drosophila* consistently occurs at specific times of the day (primarily during the morning), and this rhythmic behaviour persists under constant darkness (Xu et al. 2008). It has been shown that many organisms have evolved biological clocks to time events by metabolic processes rather than simply responding to the daily light/dark transitions. By using a circadian clock animals adjust their timing to the environment. Circadian clocks in different organisms use different sets of genes that are similar in terms of their function in the molecular feedback loops (Edery et al. 1994; Blau & Young 1999). In addition, these clock genes also influence several vital metabolic cycles and are therefore believed to play an essential role in adaptive mechanisms (Dvornyk et al. 2003). The periodic analysis of certain (metabolic) enzymes which may directly influence the entrainment of an individual with respect to different light/dark periods can be studied to understand the changes with respect to their adaptation. If flies are subjected to different light regimes for several generations it may alter their circadian rhythmicity. Maintaining flies in different light regimes is nothing but subjecting the flies to stressful condition. There are reports that *Drosophila* flies showed difference in their fitness when subjected to different light regimes (Sheeba et al. 2000; Sharma 2003). It has been shown that in *D. melanogaster* the light/dark (LD) regime affects pre-adult development time (Sheeba et al. 1999), lifetime fecundity (Sheeba et al. 2000) and adult life span (Pittendrigh & Minis 1972; Klarsfeld & Rouyer 1998; Hendricks et al. 2003; Kumar et al. 2005). Studies have shown that *Drosophila* species have maximum fertility with LD exposure and minimum with continuous dark (DD) exposure (Harini 2010). It has also been shown that in *D. melanogaster*, *D. gangotrii* and *D. jambulina* maximum time took place for complete emergence in LD, whereas in continuous light (LL) clock is fasten up and in DD clock is slow hence flies showed delayed emergence when compared to LL (Shereen & Shakunthala 2012). But there are not many reports on the effect of different light regimes on esterase isozyme profiles of *Drosophila* with respect to their circadian clock.

Esterases are complex enzymes acting on a variety of substances and are capable of hydrolysing ester bonds. In insects, esterases are involved in important physiological processes, including the catabolism of juvenile hormone (Zera & Holtmeir 1992; Shanmugavelu et al. 2000), juvenile hormone regulation (Hidayat & Goodman 1994), insecticide resistance (Morton 1993), ontogenetic development

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(Bitondi & Mestriner 1983), digestion (Argentine & James 1995), functioning of the nervous system (Villatte & Bachmann 2002), and reproduction (Karotam & Oakeshott 1993) and resistance to pesticides (Li et al. 2005). In insects, esterase genes have shown high rates of intraspecific and interspecific variation. Esterases are a very interesting group of enzymes because on one hand they are implicated in synaptogenesis, while on the other hand they are known to be involved in neuro-degeneration in adult tissue (Ahasan et al. 2009). Esterase variations have been reported in many animal and plant species, e.g. in the protozoan *Tetrahymena1*, maize (Schwartz 1960), swine (Augustinsson & Olsson 1959), man (Harris et al. 1962) and insects. Numerous enzymatic activities also have been studied in *Drosophila* (Hubby 1963; Hubby & Throckmorton 1965; Hubby & Lewontin 1966; Lewontin & Hubby 1966; Dickinson & Sullivan 1975). All these studies have suggested that the esterase isozymes exhibit high level of polymorphism in *Drosophila* and other organisms, and this polymorphism offers adaptive flexibilities to these species.

Enzyme induction by environmental cues is potentially a key adaptive process. This environment dependent response may underlie phenomena such as physiological acclimation (Magnum & Towle 1977) or morphological plasticity (Schlichting 1986). However, there are virtually no studies on enzymes in relation to the evolutionary significance of circadian rhythms. The above review of the literature has already showed that circadian rhythms have a physiological basis. Since esterases are a major group of enzymes which regulate many physiological activities, it is hypothesized that the circadian changes which occur due to different light regimes also influence the esterase pattern in regulating these rhythms. To verify this hypothesis the present work aims to analyse the effect of different light regimes in *Drosophila*. Variation in the alpha and beta esterase activities have been studied using polyacrylamide gel electrophoresis in light/dark (12L:12D), continuous light (LL) and continuous dark (DD) at 15th and 30th generations. For this study, two species of the *montium* subgroup that are closely related, i.e. *D. gangotrii* and *D. jambulina*, and the most common species *D. melanogaster*, belonging to the *melanogaster* species group, were used. The members of *montium* are heterogeneous both morphologically (Lemeunier et al. 1986) and karyotypically (Bock & Wheeler 1972; Baimai 1980; Shyamala & Ranganath 1994; Suma & Ranganath 1997; Shakunthala & Ranganath 2007). Several reports in different disciplines of biology have revealed that species closely related, as Scouras (1995) feels 'system comprising species of close phylogenetic relationship as well as species rather distant phylogenetically from each other greatly facilitate biological analysis such system is offered by the *montium* a sub group of *Drosophila*'. The purpose of using these three species is to find out the differential or species-specific responses, if any, to different light regimes.

Materials and methods

Three species, *D. melanogaster*, *D. gangotrii* and *D. jambulina* were used for the present investigation. Isozyme profiles of both sexes were studied in three different light regimes – light/dark (12L:12D), continuous light (LL) and continuous dark (DD) – for 30 generations. The flies were maintained in an environmental chamber at constant temperature of 20 ± 1°C and 75% relative humidity with the above light regimes and one group was maintained in the normal light conditions in the laboratory (LP: lab populations) but at the same constant temperature of 20 ± 1°C. This group served as control. At the 15th and 30th generations five-day-old adult flies (five males and five females) were drawn from each of the above four groups and used for the enzyme assay. The enzyme profiles of alpha esterase (α-est) and beta esterase (β-est) were analysed.

Polyacrylamide gel electrophoresis (PAGE – 7.5% native gel)

The polyacrylamide gel electrophoretic technique described by Hegde (1979) was used with appropriate modifications for slab gel electrophoresis. The sample homogenate was prepared from five adult male and female flies separately with 40 µl of 40% sucrose solution in an Eppendorf tube using a Knot's pestle. The samples were homogenized by keeping the set-up on ice and later centrifuged at 4000 rpm for 5 min at 4°C. 7.5% polyacrylamide gel was used for separation of enzyme fractions. An equal (15–20 µl) volume of supernatant was carefully loaded to each well. The first well was loaded with 1% bromophenol blue as a dye marker. After the sample application, electrophoresis was carried out at 4°C with 50 volts for 1 hour and at 60 volts till the run was continued until the tracking dye migrated the entire length of the gel. Esterase isozymes were identified in the gels by following the procedure described by Hegde (1979) using α- or β-naphthyl acetate as substrates. After the appearance of bands, the gels were photographed. Different α- or β-esterase loci were identified following the procedure described by Hegde (1979). Accordingly three esterase loci, namely est-1, est-2 and est-3, were found in the present studies each with two alleles, one fast (F) and one slow (S).

Results

The results of the qualitative analysis of the alpha esterase (α-est) and beta esterase (β-est) enzymes using polyacrylamide gel electrophoresis are provided in Table 1 and Figures 1 and 2. The figures show the banding patterns of the three species under different light regimes and generations. It has been reported that there are three loci in *Drosophila* species, designated α-est 1, α-est 2 and α-est 3 (Naseerulla & Hegde 1993; Barker et al. 1986; Sokal et al. 1987). Similarly, in the present research work *Drosophila* species showed three loci with slow moving and fast moving bands (alleles).

Table 1. Isozyme profiles of α- and β-esterases in three populations of male and female *D. melanogaster*, *D. gangotrii* and *D. jambulina*.

	α-esterase			β-esterase		
Species/light regime	*D. melanogaster*	*D. gangotrii*	*D. jambulina*	*D. melanogaster*	*D. gangotrii*	*D. jambulina*
LP	2A (2S and 2F)	1A (3S)	1A (3F)	2A (2S and 2F)	1A (3S)	1A (3F)
LD 15	1A (1S)	1A (2S)	1A (2F)	1A (1S)	1A (2F)	1A (3S)
LD 30	1A (1S)	1A (2S)	1A (2F)	1A (1S)	1A (2S)	1A (2F)
LL 15	1A (1F)	1A (2F)	2A (2S and 2F)	1A (1S)	1A (1F)	2A (1F and 2S)
LL 30	1A (1F)	1A (2F)	1A (3S)	1A (1F)	1A (2F)	1A (3S)
DD 15	2A (2S and 2F)	1A (2S)	1A (2F)	2A (2S and 2F)	1A (1F)	1A (3F)
DD 30	♂ has 1A (2F), ♀ has 1A (2S)	1A (2S)	1A (2F)	♂ has 1A (2F), ♀ has 1A (2S)	1A (1F)	1A (3S)

A = alleles.

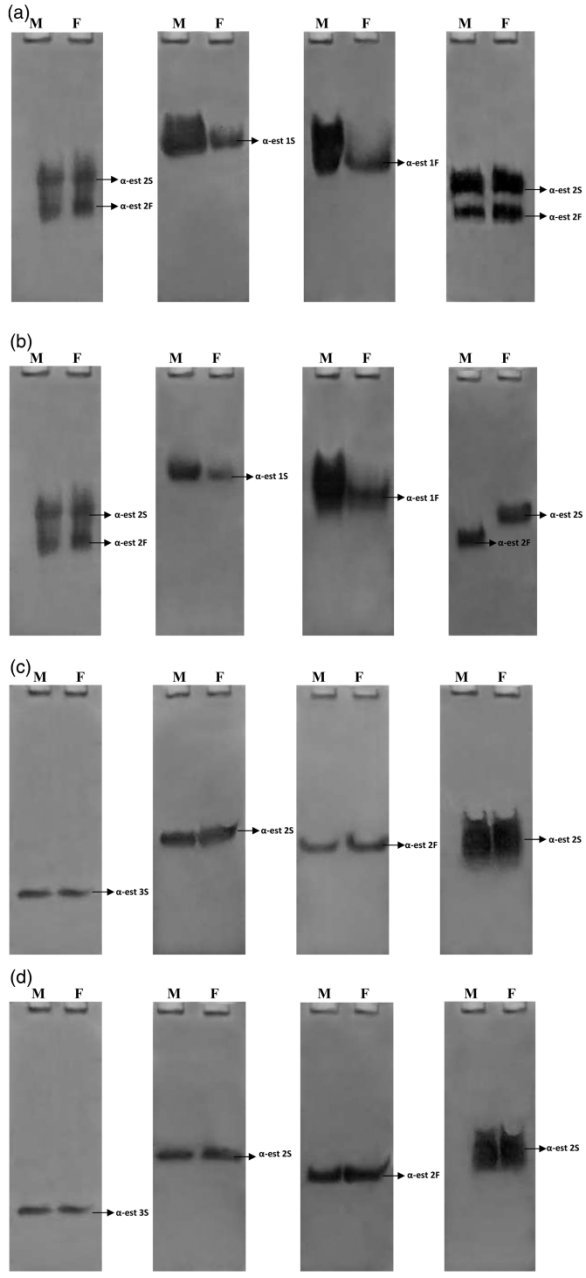

Figure 1. Alpha esterase profiles. Male and female *D. melanogaster* at (a) LP, LD 15, LL 15, DD 15; and (b) LP, LD 30, LL 30, DD 30; male and female *D. gangotrii* at (c) LP, LD 15, LL 15, DD 15; and (d) LP, LD 30, LL 30, DD 30; male and female *D. jambulina* at (e) LP, LD 15, LL 15, DD 15; and (f) LP, LD 30, LL 30, DD 30.

Alpha esterase variation under different light regimes

D. melanogaster

In LP, both the sexes of *D. melanogaster* showed an α-est 2 slow moving band (α-est 2S) and an α-est 2 fast moving band (α-est 2F). In LD 15 and LD 30 both male and female flies showed one allele each, i.e. α-est 1S, whereas in LL 15 and LL 30 both male and female flies showed single band (α-est 1F). However, in DD 15 as in LP, α-est 2S and

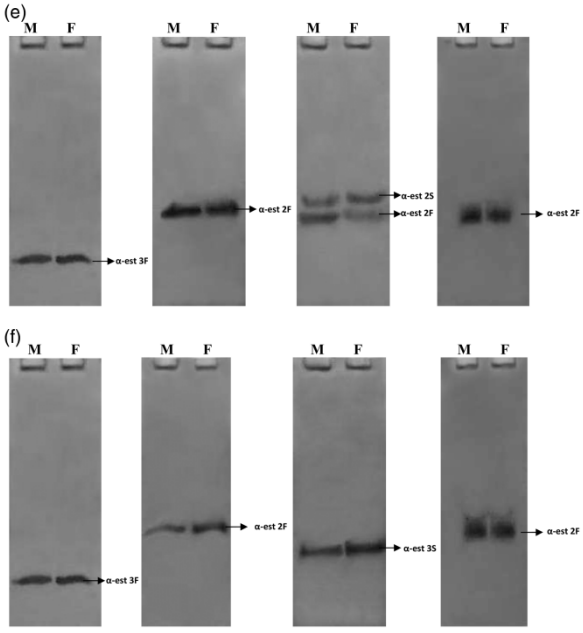

Figure 1. continued

α-est 2F were found both in males and females. At DD 30 male flies showed α-est 2F and females showed α-est 2S (Figure 1(a, b)).

D. gangotrii

D. gangotrii males and females showed α-est 3S in LP. In LD 15 and LD 30 both the sexes have α-est 2S. In LL 15 and LL 30 male and female flies showed α-est 2F, whereas DD 15 and DD 30 flies have α-est 2S (Figure 1(c, d)).

D. jambulina

In LP of this species, both the sexes showed α-est 3F. At LD 15 and LD 30 male and female flies showed α-est 2F. At LL 15 two alleles were observed (α-est 2S and α-est 2F), whereas in LL 30, α-est 3S was observed. However, in DD 15 and DD 30 both the sexes have α-est 2F (Figure 1(e, f)).

Beta esterase variation under different light regimes

D. melanogaster

Both the sexes in laboratory populations showed two alleles (β-est 2S and β-est 2F). In LD 15 and LD 30, male and female flies showed β-est 1S, whereas in LL 15, β-est 1S and in LL 30, β-est 1F were observed. However, in DD 15, β-est 2S and β-est 2F were found both in males and females as in LP. At DD 30, males have β-est 2F and females have β-est 2S (Figure 2(a, b)).

D. gangotrii

D. gangotrii males and females showed α-est 3S in LP. Both the sexes have β-est 2F and β-est 2S respectively in LD 15 and LD 30. In LL 15 male and female flies showed β-est

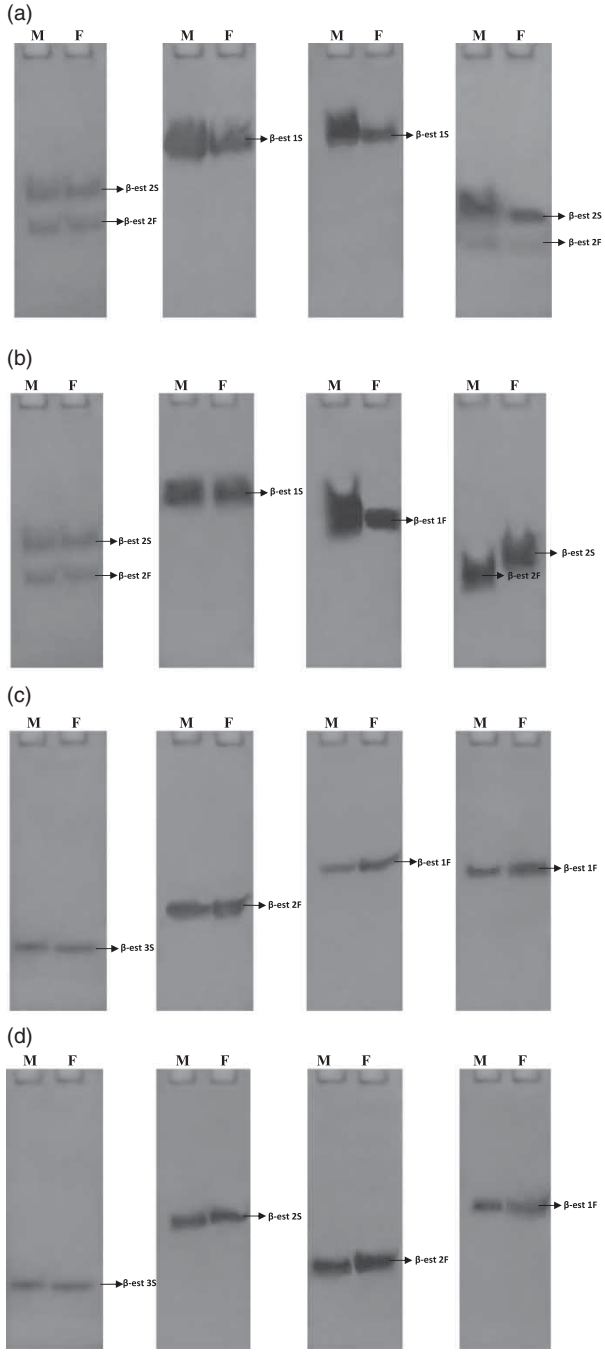

Figure 2. Beta esterase profiles. Male and female *D. melanogaster* at (a) LP, LD 15, LL 15, DD 15; and (b) LP, LD 30, LL 30 and DD 30; male and female *D. gangotrii* at (c) LP, LD 15, LL 15, DD 15; and (d) LP, LD 30, LL 30, DD 30; male and female *D. jambulina* at (e) LP, LD 15, LL 15, DD 15; and (f) LP, LD 30, LL 30, DD 30.

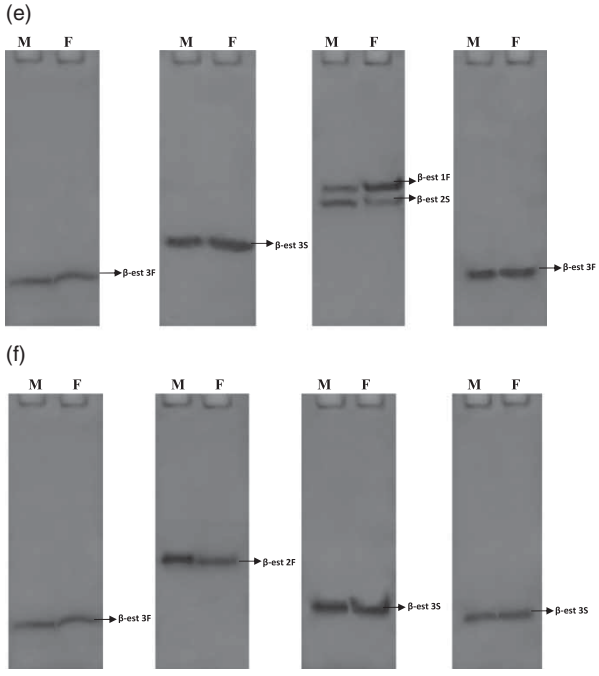

Figure 2. continued

1F and in LL 30 flies have β-est 2F, whereas in DD 15 and DD 30 β-est 1F was present (Figure 2(c, d)).

D. jambulina

In LP, both the sexes of *D. jambulina* showed β-est 3F. At LD 15 male and female flies showed β-est 3S and in LD 30 both the sexes have β-est 2F. Two alleles were observed at LL 15, those were β-est 1F and β-est 2S, whereas in LL 30 only one allele was found, i.e. β-est 3S. However, in DD 15, β-est 3F and in DD 30, β-est 3S were present (Figure 2(e, f)).

Discussion

Esterases are a heterogeneous group of enzymes catalysing various esterification reactions. They are classified as non-glucose metabolizing enzymes (Gillespie & Kojima 1968). Studies have demonstrated that the genetic loci encoding the synthesis of esterases have high variability (Naseerulla & Hegde 1993). In the present investigation, there were variations in both alpha and beta esterase patterns in all three species employed, thus this study provides additional testimony for the esterase polymorphism that has been already reported. It has been noticed that there were three alpha esterase loci in *Drosophila* species which were designated as α-est 1, α-est 2 and α-est 3. Similarly there were three beta esterase loci which were designated as β-est 1, β-est 2 and β-est 3. Each locus in turn had two alleles represented by a slow moving (S) and fast moving alleles (F). Investigations on the changes in enzyme activity in space and time among and within populations of Drosophila (Barker et al. 1986; Sokal et al. 1987) have been carried out. The overall number of esterase loci and alleles noticed in all the three species were also found in all these studies. However, the loci or the allele expressed at given light regime in these species exhibited variation.

The present study also shows that different light regimes change the pattern of expression of esterase alleles. For example, the pattern of expression of alpha and beta esterases in *D. melanogaster*, *D. gangotrii* and *D. jambulina* varied in different light regimes and at 15th and 30th generations. According to Sharma (2003) the changes in the light regime affect the physiology and also the rhythm of the fly. These physiological changes have reflected in terms of α-esterase or β-esterase expression by switching over from one locus to another over generations. It was also observed that maintaining the flies in different light regimes can alter their allelic pattern. For example in *D. jambulina*, for α-esterase, both at LL 15 and LL 30 different alleles were found when compared to other light regimes, however for β-esterase there were no similarities in allelic patterns in the light regimes. It has been shown that environmental changes can reduce the fitness of an organism (Hoffmann & Parsons 1991). In the present situation subjecting the flies to different light regimes has altered the pattern of expression of alleles and hence their enzyme activity. There was no change in the expression of alleles from 15th to 30th generations in all light regimes except in LL, wherein there was change from 15th to 30th generation. In *D. jambulina* allelic variation was noticed in all light regimes when compared to LP. Flies maintained in different light regimes and generations showed variation in the expression of their alleles with respect to alpha esterase and beta esterase. But there were also certain similarities in alleles found at 15th and 30th generations within the same species.

We observed variation in expression of alleles in the form of presence or absence of a given band in different light regimes. Some alleles were expressed in some light regimes but not in another regime. This shows that the different light regimes affect the expression of esterase alleles. As mentioned in the introduction, biological rhythms are endogenously generated self-sustaining mechanisms which are influenced by environmental stimuli such as light and feeding (Schibler 2005). The organism is able to quickly adjust or adapt to changing conditions by making appropriate physiological changes. The physiological change which is brought about by the change in the rhythm is evident in the present study because the esterase pattern was different in different light regimes. Thus this observation also confirms the hypothesis that circadian changes which occur due to different light regimes also influence the esterase pattern in regulating these rhythms.

The three species, *D. melanogaster* and the two species of the *montium* group showed differences in the expression of alleles at different light regimes. Although the *montium* subgroup is closely related to the *melanogaster* species group there was variation in terms of banding. This

shows the differential response of different species to the light regimes. There was species specific difference which has some bearing on the general activity of these flies. *D. melanogaster* is a cosmopolitan domestic species and the flies are quite active with high fecundity and fitness in most environmental conditions (Bock & Wheeler 1972). However, in the *montium* subgroup, both *D. gangotrii* and *D. jambulina* coexist in nature, and there were no similarities in their expression of alleles at different light regimes. However, these two species are more closely related to each other than to *D. melanogaster*. So the present study showed that the two *montium* species are closely related and are similar in their response to different light regimes.

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