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Abstract

Animals can cope with spatiotemporal variation in their environment through mobility and selective habitat choice. Intra-specific variation in habitat choice has been documented especially for host plant preferences and cryptic habitat selection in insects. Here, we investigated the genetic variation in light sensitivity and light-dependent habitat choice in the eyeless Collembola *Folsomia candida* with a choice test under four different lighting conditions (control dark condition, two simulations of undergrowth natural light conditions and red light). We tested twelve clonal strains from diverse geographical origins that are clustered in two evolutionary clades with contrasting fast or slow life-

history strategies. The clones differed in their mean movement probabilities in the dark treatment. These differences were related to the two different phylogenetic clades, where fast-life history clones are on average more mobile than slow-life history counterparts as predicted by the 'colonizer syndrome' hypothesis. We found behavioural avoidance of light in the three light conditions. Moreover, photophobia was stronger when the simulated light spectrum was brighter and included non-red light. Photophobia was similar among all clonal lineages and between the two clades, which suggests that this behaviour is a shared behavioural trait in this species. We discuss the use of light as an environmental cue for orientation, displacement and habitat choice under natural conditions.

Keywords

Folsomia candida, Collembolan, light sensitivity, eyeless species, mobility, intraspecific variation

Introduction

Animals use various environmental cues for habitat choices, and different individuals from a single species may show contrasting habitat preferences depending on their sex, stage, size and genetic background for instance (Stamps, 2001: Matthysen, 2012). This intra-specific variation of behavioural responses may influence a wide range of eco-evolutionary processes (Sih et al, 2012; Ronce and Clobert, 2012; Edelaar and Bolnick, 2012). Inter-individual differences in habitat choice behaviours may be genotype-dependent and related to differential performance in specific habitats or niches (Sih et al, 2012; Edelaar and Bolnick, 2012; Hawthorne and Via, 2001; Cousyn et al, 2001; De Meester, 1996; Jaenike and Holt, 1991). Genetic variation in habitat choice behaviour is

common in animals and has been well documented for host plant preferences and cryptic habitat selection in insects (reviewed in Jaenike and Holt, 1991).

One of the cues animals use to select their habitat is light which animals may be positively or negatively attracted to. For example, negative phototaxis acts as a predator-avoidance mechanism in some aquatic organisms (De Meester, 1996; Cousyn et al, 2001; Michels and De Meester, 2004; Borowsky, 2011) and may help some soil organisms that are very sensitive to relative humidity to prevent desiccation by looking for deeper and more humid soil layers (Salmon et al, 2014; Salmon and Ponge, 2012). Nevertheless, different individuals or populations within the same species may present different phototactic preferences. For example, clonal populations of *Daphnia magna* (a zooplancton species) exposed to high levels of predatory pressure are more photophobic than clonal populations less exposed to predation (Cousyn et al, 2001; De Meester, 1996). In general, differences in phototaxis may have a heritable, genetic basis (e.g., Markow and Smith, 1977; De Meester, 1996; Cousyn et al, 2001) or could be the result of non-genetic, phenotypic plasticity and personality (is this too anthropomorphic for Daphnia or springtails?) differences among individuals (e.g., Kain et al, 2012). Quantifying sources of variation in phototaxis is therefore important to understand the evolution of this widespread behavioural trait. Here, we investigate the genetic variation in light sensitivity and light-dependent movement behaviour in the eye-less springtail *Folsomia candida* Willem 1902 (Collembola, Isotomidae), an hemi-edaphic and cosmopolitan soil organism inhabiting various habitats such as caves, forest litter and man-made habitats (Fountain and Hopkin, 2005).

The degeneration or even loss of the visual system is a convergent and frequent evolutionary phenomenon in soil-dwelling and cave animals (Christiansen, 2005). Nevertheless, even eyeless and eye-reduced species often retain some sensitivity to the ambient light level through extra-ocular photoreceptors (EOP, Taddei-Ferretti and Musio, 2000; Ullrich-Lüter et al, 2011), which are useful for the maintenance of circadian rhythms (Friedrich, 2013) or for orientation and habitat choice (Timmermann and Plath, 2008; Borowsky, 2011). Indeed, previous works strongly suggest that *F.*

candida is sensitive to light despite being eyeless. In choice-test experiments, *F. candida* avoids UV light moving to warmer locations exposed to white light, prefers darkness over cool white light (Fox et al, 2007), and displays a dose-response avoidance of UV-B light relative to darkness (Beresford et al, 2013). Yet, to our knowledge, no study has examined the wavelengths of maximum sensitivity of the ocular or extra-ocular photoreceptors (EOPs)of these animals (Barra, 1971; Jordana et al, 2000; Fox et al, 2007). In true insects (Pterygota), few species are able to detect wavelengths longer than 600 nm (red light), which suggests a red-blind common ancestor (Briscoe and Chittka, 2001). In addition, the behavioural tests mentioned above could not always prevent confounding effects of differences in temperature or humidity associated with the lighting treatment. This is of great importance, as *F. candida* needs a relative humidity close to saturation (Holmstrup, 2002; Waagner et al, 2011) and is very sensitive to temperature (Boiteau and Mackinley, 2012, 2013).

Although sexual reproduction exists in some populations of *F. candida* (Frati et al, 2004), this species is generally recognized as asexual, and most studies using *F. candida* as a model species have used parthenogenetic lineages (Fountain and Hopkin, 2005). Earlier studies on several parthenogenetic lineages have uncovered substantial intra-specific genetic and morphological polymorphism (Chenon et al, 2000; Tully et al, 2006; Tully and Potapov, 2015). Intra-specific diversity is organised in two major evolutionary clades (Tully et al, 2006; Tully and Potapov, 2015), and life history studies have shown that two contrasted biodemographic strategies evolved along the divergence of these two clades (Tully and Ferriere, 2008; Tully, 2004; Tully and Lambert, 2011; Mallard et al, 2015). One clade has a high reproductive potential: when sufficient food is available, these springtails produce on average larger clutches than the ones from the other clade (Tully and Ferriere, 2008), but they have shorter mean lifespans than the less fecund clade and also reach a smaller adult body size (Tully and Ferriere, 2008; Tully and Lambert, 2011; Mallard et al, 2015). These two groups of clonal lineages fit well to the typical slow (A) and fast (B) life history syndromes (or r-K life histories, see Reznick et al, 2002). But, until now, the ecological conditions

in which they have evolved and the time elapsed since the divergence of the two clades remain to be determined. Intra-specific variation in habitat choice behaviour and mobility has so far neither been examined in this species nor in other Collembola. Instead, the few works that relate the habitat preference or distribution and colonization ability of Collembola with their morphological and life history traits are focused on the study of collembolan community composition (Salmon et al, 2014; Ponge and Salmon, 2013, Huebner et al, 2012; Salmon and Ponge, 2012; Ponge et al, 2006). Intraspecific variation in phototactic behaviour and life history traits has been well investigated in *Daphnia magna*. In this species, positive phototactic clones present a fast life history strategy whereas negative and intermediate phototactic clones present a slow life history strategy (e.g., De Meester, 1994).

We tested if springtail clonal variation in light-dependent habitat choice exists using an experimental setup to control the lighting conditions while maintaining constant temperature and moisture. We tracked springtail movements under this setup to quantify their spatial preference for shaded versus illuminated areas as a measure of the habitat choice behaviour. We first tested whether *F. candida* can use light as an environmental cue for habitat choice under different lighting conditions, including natural shaded and sunny understory spectra and an artificial red-light spectrum. We measured springtail sensitivity to long wavelengths because these wavelengths are dominant under the forest canopy (Smith, 1994) while red and far red are the principal wavelengths that penetrate the soil (Bliss and Smith, 1985). We hypothesised that *F. candida* should not detect or react to red and far-red light *per se* if the incapability to detect these wavelengths was a shared condition of most true insects and Collembola, even though most springtails are sensitive to heat generated by red light (Briscoe and Chittka, 2001). We further studied the sensitivity to light of twelve clonal lineages of *F. candida* including eleven lineages belonging to the two evolutionary clades described earlier (Tully et al, 2006; Tully and Ferriere, 2008; Tully and Lambert, 2011; Tully and Potapov, 2015). We addressed the following questions: Do light sensitivity and habitat choice behaviour vary between clonal lineages, as has been found in other taxa (Jaenike and Holt, 1991;

De Meester, 1996; Cousyn et al, 2001)? If such clonal variation exists, how is it organized relative to the phylogenetic clades and what are the links between the behavioural responses and the main life history strategies of each clade? We predicted that lineages from the slow life history group would be more photophobic (De Meester, 1994; De Meester, 1995), given that photophobia is likely to be associated with life in more stable habitats, which usually selects for a slow life history (Pianka, 1970; Reznick et al, 2002).

Materials and methods

Maintenance and origin of the studied springtails

We used twelve clonal lineages of the Collembola *Folsomia candida* labelled AP, BR, BV, DK, GB, GM, HA, ME, PB, TO, US and WI (Tully et al, 2006). Information about the phylogenetic relationships and habitat and geographical origin of all strains except ME can be found in previous studies (Tully et al, 2006; Tully and Potapov, 2015). Clones AP, BV, BR, GB and HA belong to the "slow clade" A while the "fast clade" B comprises the clones DK, GM, PB, TO, US and WI (Tully and Ferriere, 2008). The new clone ME was collected in November 2013 from some decaying wood beams into an abandoned man-made tunnel in the Mercantour French National Park (South-East of France, 44° 7.026'N, 7° 16.727'E, 1530 m). The life history strategy of this clone and its phylogenetic relationships with the other clones are currently unknown.

All clonal populations were reared in similar conditions in polyethylene vials (inner diameter 52 mm, height 65 mm) filled with a 30 mm layer of plaster of Paris mixed with Indian ink to increase visual detectability of individuals (Tully and Ferriere, 2008). Populations were kept in incubators at 20°C (± 0.5°C) in the dark and fed with pellets of a mixture of agar and dried yeast (Tully and Ferriere, 2008). We established synchronised populations of each clone in the same way and at the same time by transferring 10-12 randomly chosen adult females from stock cultures to new culture vials. Females were transferred to new vials every week and old vials were kept at 20°C for laid eggs to hatch since this temperature is optimal for all lineages. Then, vials were checked weekly for the presence of new-borns. If new-borns were detected, *ad libitum* food was

provided weekly to ensure optimum body growth. Therefore, the age of the experimental animals was known with a one-week accuracy. However, due to the low fecundity of some clones or accidental flooding in some vials, we had to add medium size (~1.4mm) adults of unknown age taken from the stock cultures in order to keep balanced samples for all clones and treatments (see Table S1 for details on sample size and individual characteristics).

Measurement of natural light conditions

At midday of a sunny day in June 2013, we measured 23 light spectra under the canopy of a temperate forest located in the Foljuif field station, 80km South of Paris, France (48°17'13.96"N, 2°40'40.34"E), where populations of *F. candida* have been previously found. We recorded 19 spectra in the shade and four spectra on sunspots in the undergrowth. The climate conditions and plant community structure of this forest have been studied extensively (Blandin et al, 1980). We used a handheld spectrometer parameterized for absolute irradiance measurements in the range 200-850 nm (Jaz Series, JAZ-ULM-200, Ocean Optics, USA). We measured the incident light with a cosine-corrected probe (180° field of view) after dark calibration and converted data into units of irradiance spectrum ($\mu W.cm^{-2}$) using factory calibration of the spectrophotometer. Measurements were taken at ground levels under the canopy of different tree and bush species, as well as under sunspots under the trees and into tree stump holes to sample different light spectra that natural populations of springtails are likely to encounter aboveground.

Simulation of environmental conditions

Artificial light and constant climate conditions were simulated in a controlled environment laboratory at the CEREEP EcotronIleDeFrance (Saint-Pierre-les-Nemours, France). We used one 13 m^3 controlled environment chamber of the Ecolab, where climate (temperature, relative humidity, rainfall) and lighting conditions can be simulated (Verdier et al, 2014). In the centre of the chamber, we installed vials for the characterisation of photophobia in springtails (see below) on a controlled water table to maintain constant humidity and temperature conditions in the vials during the observations. Controlling for constant humidity and temperature was essential to test

exclusively for the effect of light, since slight changes in humidity and temperature could modify the springtails' behaviour and thus affect their apparent or real photophobia (Holmstrup et al, 2002; Boiteau and Mackinley, 2012). Atmospheric temperature in the chamber was maintained at 20ºC (± 0.1°C) and temperature of the water table was set at 10ºC. We used a cold water table to keep the temperature inside the vials slightly lower than the air temperature to prevent condensation on the transparent lid that covered the vials (Figure 1). The temperature measured inside the vials was 19ºC and the relative humidity was 93% (DS-1923 iButton loggers) irrespective of the test conditions.

Light was provided by means of modular LED (light emitting diodes) arrays (n=40) allowing to turn on or off cool white LEDs (n=15 per array) and five different LED types with maximum emission wavelengths in the UV (370 nm, n=3), green (520 nm, n=2), red (660 nm, n=5), far red (740 nm, n=7), and infrared (840 nm, n=3). Thus, up to 36,864 combinations of LEDs parameters can be programmed. To automatize the simulation of the most appropriate lighting conditions, we wrote a procedure in R (R Development Core Team 2012) to calculate the sum of squared distance between the reference spectrum and the simulated spectrum, and to select the combination of parameters that minimised this sum. We simulated the "average undergrowth" light spectrum by producing a spectrum which best matched the mean spectra of the 23 light measurements made at various places on the forest floor (see Figure 2a and Table S2 provided as supplementary information). We also produced a "maximum undergrowth spectrum", using the four measurements made on sunspots, which should correspond to the strongest undergrowth light conditions that litter-dwelling springtails are susceptible to be exposed to in this forest (Figure 2b and Table S2). We expected a stronger light-avoidance response under this condition compared to under the mean undergrowth light spectrum. In addition, we simulated a "red light" spectrum whose power was close to that of the mean undergrowth light spectrum (Figure 2a and Table S2). This red light spectrum has two irradiance peaks (red at 660 nm and far red at 740 nm) and has no UV light that could promote photophobia in springtails (Fox et al, 2007; Beresford et al, 2013). We further

compared these three lighting conditions with a control treatment where the room was maintained in the dark. This condition was used to verify whether the experimental set-up in itself could affect the behaviour of the springtails and to compare the mobility of the different clones in the dark.

Experimental protocol

The dark, average undergrowth and red light spectra were tested in June 2014, and the maximum undergrowth spectrum was tested in December 2014. We used 36 individuals of each clonal lineage in each light treatment (n = 1728), and six individuals per clone were tested every day. Springtails were photographed to measure their length (mean = 1.41 mm, range =0.93-2.19 mm) and kept isolated without food in rearing vials the day before. These individuals were transferred the next morning to new rearing vials wrapped with a thin flexible black plastic sheet, and only exposed to light from above. Each plastic box was filled with plaster of Paris and had a hole in the bottom to ensure that the plaster can be kept constantly wet to saturation (Figure 1). An opaque black piece of plastic covered half of the vial's opening and descended vertically along the centre of the vial down to 3 mm above the plaster level, in order to maintain in the dark half of the vial while allowing the springtail to move freely between the two halves of the vials. Preliminary experiments showed that in total darkness springtail behaviour is very sensitive to drought and slight humidity differences between the two sides of the vials. The vials were thus covered with transparent lids to prevent any desiccation. When being transferred, animals were separated randomly in two groups of same size and released in the bright and dark sides at the beginning of the experiment (here called 'position') respectively, such that the proportion of animals in each position was exactly 50% for each clone and treatment. One observer (MGR) studied the springtails' movements between the two sides of the vials by recording the position of each animal every hour during six hours (from 9 am to 5 pm). For the control dark treatment, we observed the springtails' positions as quickly as possible using a soft light head torch to minimise disturbance. At the end of the experiment, we inspected all vials to check that no individual had escaped or deceased to ensure valid observations when the springtails did not move.

Statistical analyses

We used the software R (R Development Core Team 2012) for the statistical analysis and to produce the graphs. The raw data and the R-scripts of the analysis, and figures are provided as supplementary information. We calculated the movement (or transition) probabilities (the probability that an individual has moved from one side to the other side of the vial after one hour), using pairs of consecutive observations of the same individual to form a binomial variable which equals zero when the individual stayed in the same half of the vial and equals one when the animal had moved to the other. This binomial variable was analysed with nested generalised linear mixed models (GLMMs) for repeated measurements approach with a binomial error distribution and logit link function, using treatment (light spectrum type), position (dark or light) and clonal group as the fixed factors, and individual as the random factor. Observations were thus clustered in individuals and individuals were nested within clones. In the full model, we analysed all main effects as well as two and three-way interactions. We implemented the full model with the *glmer* procedure in package *lme4* for R 3.1.2 (Bates et al, 2012; R Development Core Team 2012) and tested for the significance of fixed effects with Chi-square tests. Since the three-way interaction Spectrum*Position*Clone was significant (see Table S3 provided as supplementary information), we analysed each treatment independently to get a better understanding of the behavioural differences among clones under each treatment.

For subsequent analyses we further included the body length of each individual in the analysis to test for a size effect on individual movement probability. Since mean body length can vary substantially among clones (Mallard et al, 2015), we calculated for each treatment the corrected body lengths as the sum of the overall mean body length and the body length residuals computed here as the difference between individual lengths and mean length of their clone. This "corrected body length" was then used to study the effect of body length while controlling for differences among clones. The full models included position, clone and corrected length as fixed factors, the two-way interactions and individual identity as the random factor. We used a backward stepwise

selection to get final models that only included significant factors and interactions. We also report full models in Table S4 following Forstmeier and Schielzeth's recommendation (Forstmeier and Schielzeth, 2011). For the full and final models, we calculated marginal and conditional R^2, which quantify the amount of variance explained by the models (Nakagawa and Schielzeth, 2013). We additionally performed GLMMs with clade, position and their interaction as fixed factors, and clone and individual within clone as nested random factors to test for phylogenetic variation. The clone ME, whose evolutionary clade has not yet been characterized, was excluded from these analyses.

Results

In the homogenous dark environment, the mobility of springtails was not influenced by their position in the vial (Table 1; Figure 3) and was independent of their body length. The individual mobility (average transition probability) differed significantly among clones (Table 1, Figure 4a), and this variability was mostly due to difference between the two evolutionary clades (χ^2_1= 15.86, $p<0.001$). In complete darkness, the clones with a fast life history (clade B) had on average a higher propensity to switch between sides (0.44 per hour) than clones with a slow life-history strategy (0.34, Fig 4a).

Contrary to our expectations, *F. candida* proved to be slightly sensitive to red light (Table 2), independently of their length: springtails preferentially moved from red light to dark (0.46 per hour) than from dark to red light (0.42, see Figure 3). Clones also differed in their mean mobility, but these differences were not due to clade (χ^2_1 =0.07, p = 0.79, Figure 4b).

Springtails showed photophobia when exposed to average undergrowth light: they moved from the light to the dark side significantly more often than in the reverse direction (0.43 versus 0.37, see Fig 3 and Table 3). The different clones (χ^2_{11}= 18.09, p=0.08) and the two clades (χ^2_1= 0.40, p=0.40) had similar movement probabilities (Figure 4c and Table 3). In this light condition, movement probability varied with body length depending on position: while in the dark compartment the length did not influence mobility, but when exposed to light, smaller than average

springtails were more prone to move than longer ones (Figure 5a). When exposed to maximum undergrowth light, springtails moved away on average more often (0.45 per hour) than when they were in the dark side of the vials (0.36, see Figure 3 and Table 4). Differences among clones (χ^2_{11}=15.67, p=0.15) and clades were not significant (χ^2_1 =1.04, p=0.31, Figure 4d), and there was a non-significant trend (Position* Corrected Length: χ^2_{11}=1.22, p=0.27) for longer than average individuals to move less often than smaller individuals, as observed in the average undergrowth light treatment (Table 4). However, there was a significant interaction between effects of clone and of corrected length: for most clones, size matters little and smaller individuals tended to move more, while for clone GB the mobility was positively influenced by relative length (χ^2_{11}=21.49, p=0.03) (Figure 5b) Clone GB was characterized by the slowest life history strategy.

Discussion

Limits of the experimental protocol

Our experimental protocol allowed us to study the behaviour of isolated individuals in darkness and under three light conditions while maintaining constant temperature and humidity. As the springtails' mobility is very sensitive to these environmental factors, this represents a significant improvement to test for photophobia.

Differences in temperature between the illuminated and dark sides of the boxes fell within the measurement error of our thermometer devices (< 0.5°C). Thus, even though we cannot strictly exclude that the bright side may be slightly warmer than the dark side, the potential temperature difference was so small that we could not detect it. Thus we interpret our result as a light sensitivity but one has to remember that temperature gradients could also play a role. For instance, temperature receptors such as micro setae on the antennas or legs may help springtails to perceive small temperature gradients, in particular when doing 'turning' movements (loops), a frequent behaviour when in an unknown environment (Bengtsson et al, 2004),

In addition, our experimental design is not suited to know how many times springtails moved between sides during the one hour census interval. Our estimations of transition probabilities are

based on the hypothesis that each springtail does no or only one move in an hour, which may be wrong given that some individuals may have moved multiple times between two censuses. Future studies should track more continuously individuals through space to obtain better estimates of dispersal distances and capacities.

Light sensitivity and its ecological significance

We found that, despite being eyeless, *F. candida* is slightly but significantly sensitive to light. Photophobia was detected in the presence of the three light sources, including two light spectra chosen to mimic natural conditions encountered by springtails. Hence, this study confirms earlier general findings of negative phototaxis in this species (Fox et al, 2007; Beresford et al, 2013; Boiteau and MacKinley, 2014; Salmon and Ponge, 1998). But our study goes one step further by showing that the intensity of photophobia response varied depending on the light spectrum: Light avoidance by springtails increased from the red light spectrum at an intensity equivalent to that of the average light spectrum under the canopy, to average undergrowth spectrum, and to maximum undergrowth spectrum. *F. candida* is thus sensitive both to light intensity and to its quality. This suggests that despite being blind, these springtails can use light as an environmental cue for orientation, movement and habitat choice under natural conditions. Light avoidance behaviour was not very strong, because individuals also moved from the dark to the illuminated side of the vials and switched their position frequently. This may be due to the fact that the intensity of the light spectrum tested was low compared for example to direct sunlight, but could also be related to the small spatial scale of the setup and the short time scale of our tests.

Multiple examples of eye-reduced and eyeless species that retain brightness and colour sensitivity exist (e.g. Ramirez et al, 2011; Friedrich, 2013). Extra-ocular photoreceptors (EOP) or non-visual photoreceptors can be responsible of light sensitivity when eyes are absent. EOP exist in the form of dermal photoreceptors in invertebrates (Tosini and Avery, 1996; Binder and McDonald, 2008; Xiang et al, 2010; Ullrich-Lüter et al, 2011), the sixth caudal ganglion in decapods (Wilkens and Larimer, 1976; Larimer, 1966), genitalia (Arikawa et al, 1980) and neurons of the optic lobes in

butterflies (Lampel et al, 2005), and photosensitive cells in the hydra (Taddei-Ferretti and Musio, 2000). *F. candida* lacks external eye facets and any other external eye structures as revealed by scanning electron microscopy (Fox et al, 2007). Whether *F. candida* is only facet-less but has internal photoreceptors where eyes should be or whether it uses other types of extra-ocular photoreception such as a dispersed photoreception dermal sense (Ramirez et al, 2011) or neural photoreception (Lampel et al, 2005; Wilkens and Larimer, 1976), remains an open question.

Photophobia may help springtails to avoid open spaces when they forage in the litter close to the surface, and together with positive geotaxis (Boiteau and MacKinley, 2014), it may serve for orientation in vertical movements. Being able to avoid open spaces and the surface of the litter may provide several benefits. First of all, all clonal lineages tested here were non-pigmented and thus little protected against the deleterious effects of UV radiations. Long-term (two weeks) exposure to UV-B light increases mortality and causes DNA damage in this species (Beresford et al, 2013). Behavioural tactics of habitat selection for dark sheltered zones or deeper soil layers can help springtails to escape from potentially dangerous UV-radiation. This may also explain why unpigmented or slightly pigmented Collembola are preferentially distributed in edaphic or hemi-edaphic rather than epigeal habitats and in woodlands rather than in grasslands (Salmon et al, 2014; Salmon and Ponge, 2012). However, the photophobia of *F. candida* cannot be exclusively related to UV-radiation avoidance, since *F. candida* was also sensitive to red light. Long-waves are dominant wavelengths under the forest canopy (Smith, 1994), and thus sensitivity to these wavelengths could be related to a more general aboveground avoidance. Moreover, as ambient light level is often correlated with other climatic factors (notably temperature and humidity), photophobia is useful to target deeper, more humid and more stable microhabitats preferred by most Collembola species, especially those that are the most vulnerable to desiccation (Salmon et al, 2014; Salmon and Ponge, 2012). Another explanation is that photophobia may indirectly help springtails to avoid predators. Being devoid of compound eyes *F. candida* probably cannot form images and thus cannot see approaching predators (Hauzy et al, 2010), as it is the case of the sister eyeless species *Folsomia*

fimentaria (Baatrup et al, 2006). This species is thus very vulnerable in illuminated environments to visually active-chasing predators such as hunting ground-beetles, some of which are ferocious predators of springtails (Ernsting, 1977).

Intra-specific variability in movement and light sensitivity

In general, smaller individuals were more mobile than larger ones and we found that smaller springtails exposed to light were also more likely to seek refuge in the dark side than the larger ones in the average undergrowth light treatment. The risk of staying in an illuminated environment may not be the same for large and small individuals. For example, it has been shown that the carabid *Notiophilus biguttatus* – a common visual hunter and voracious predator of springtails - preys more efficiently on small rather large Collembola (Ernsting and Mulder, 1981). This interpretation is tenuous given that the effect of body size on movement was small, variable across clones and not repeatable across light treatments. These variable effects of body size are difficult to explain. The intensity of photophobia may also depend on other individual state parameters such as the nutritional status or the phase in the moulting and oviposition cycle. There could also be a trade-off between the preference for safer dark deep zones and the need for foraging and dispersal at the illuminated surface (Dromph, 2003).

We searched for clonal variation in both the mean movement propensity and in the light sensitivity of movement. We first found that the various lineages moved differently in the dark: the average transition probabilities varied from 0.2 to almost 0.5 depending on the lineages. Quite remarkably, this genetic diversity was structured between the two previously described phylogenetic clades: clones from the clade characterised by a fast life-history strategy had on average higher transition probabilities than those with slow life-history strategy, suggesting that they move faster and can potentially disperse over longer distances in natural conditions. Even though we did not measure dispersal *per se* (defined as the movement of individuals or propagules that leads to gene flow, see Clobert, 2012), mobility or exploratory activity has generally been positively correlated to dispersiveness and can thus be used as a rough proxy for dispersal in natural

populations (Ronce and Clobert, 2012). The higher movement propensity of the fast life-history clade agrees with the evolutionary scenario of the 'colonizer syndrome' where fast life-history species or populations are more dispersed than their slow life-history counterparts (Ronce and Clobert, 2012). The combination of increased dispersal, high fecundity but short lifespan and low competitive ability allows fast individuals to colonise new habitats more efficiently. Individuals with reduced movement propensity, low fecundity and high competitive ability perform better at the core range and in stable habitats, where populations have reached their carrying capacity and where intraspecific competition is strong (Burton et al, 2010). Future studies should assess whether differences in short-distance movement probabilities translate into clonal differences in long-distance movement behaviour and therefore dispersal distances.

Studies on the intraspecific variation of phototaxis are scarce but differences in phototaxis among genetically distinct lineages have been reported in several species. It has been shown that several *Daphnia* clonal populations differ in their photophobia in response to contrasting predatory pressures (Cousyn et al, 2001; De Meester, 1996). Artificially selected *Drosphila* strains also differ in their phototactic preferences both in quality and degree as a result of different genotypes and the presence of specific mutations (Kain et al, 2012). But contrary to these findings, we did not find any significant differences in our model organism in light sensitivity among clones and clades for the two undergrowth spectra, including when springtails were exposed to the brightest light spectrum. This could indicate that photophobia is a shared, evolutionary rigid behavioural trait in this species. It also implies that other studies that failed to find intraspecific variation in phototaxis may be missing in the published bibliography.

In addition, when exposed to light, the genetic differences in movement behaviour observed in the dark vanish. Our observations also suggest that moving from one side of the vial to the other side in homogeneous dark conditions or in heterogeneous light conditions is associated with different underlying behaviours: exploration for the former and escape for the latter. This is also

supported by the fact that body length did not affect movement in the dark while small individuals were found to be slightly more mobile than larger ones in the heterogeneous environments.

Acknowledgments

We want to thank to the staff members of the EcotronIleDeFrance, especially Simon Chollet, Mathieu Llavata and Florent Massol. This research was supported by the Centre National de la Recherche Scientifique and a grant from the Ecole doctorale Diversité du Vivant at the Université Pierre and Marie Curie to M.G.R. (contract 859/2013). This work was made possible thanks to the CNRS Research Infrastructure Ecotrons supported by the Conseil régional d'Ile-de-France (DIM R2DS I-05-098/R and 2011-11017735) and the Agence Nationale de la Recherche grant of the "Investissements d'avenir" program (ANR-11-INBS-0001 AnaEE France).

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Figure legends

Figure 1. **a)** Photographs of the experimental boxes. Boxes were wrapped with a thin flexible black plastic sheet (1), and only exposed to light from above. Boxes were covered with a transparent plastic lid (2) to prevent desiccation and disturbance due to airflow. The arrow shows the gap between the plaster of Paris floor (3) and the opaque black piece of plastic (4) that descended vertically along the centre, so that springtails could move freely between each side of the box. **b)** Photograph of the LEDs modules.

Fig 2. Light conditions used during the experiment. a) Natural and simulated average under-canopy spectra and experimental red light spectrum. The three spectra have equivalent total power. b) Natural and simulated sunspots under the canopy spectra. The two spectra have equivalent total power.

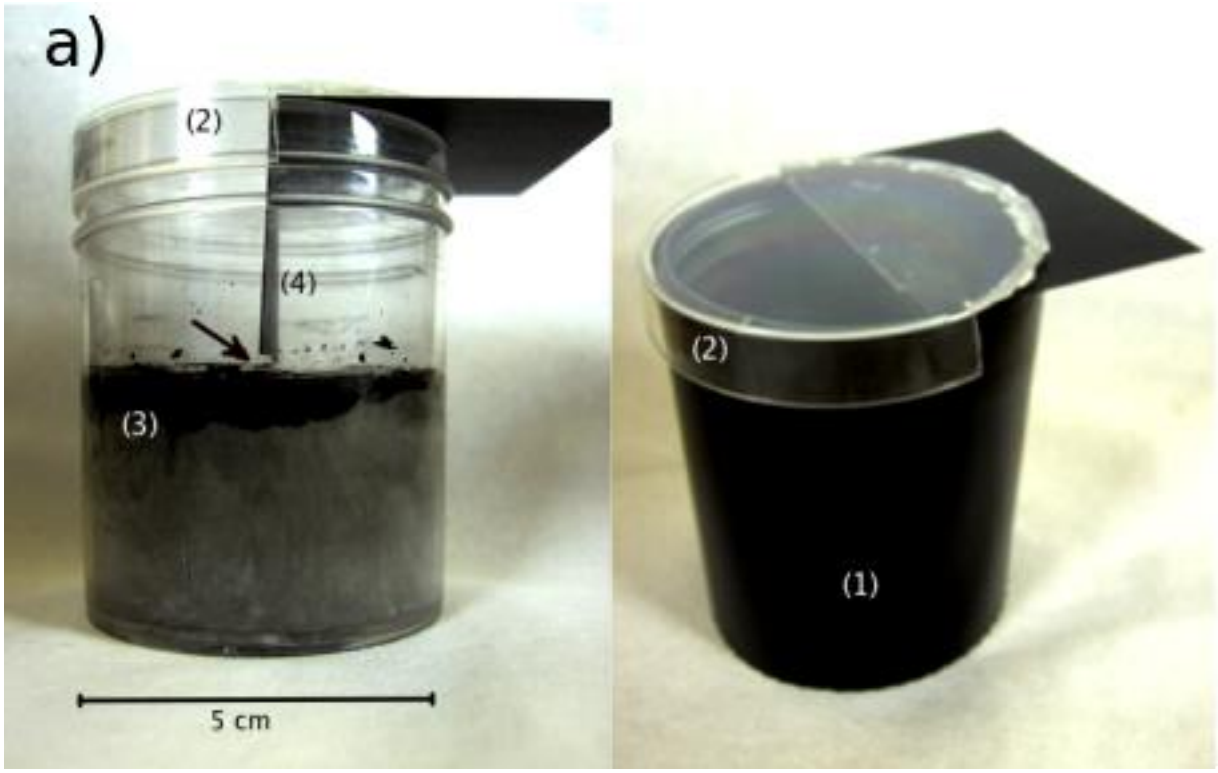
Fig 3. Mean movement probability (probability that a springtail has moved between the two sides of the test boxes after one hour ±95% CI) for each light condition of the experiment according to the initial location of the springtail (black symbol: the individual is located at the dark side, white symbol: the individual is located at the illuminated side below the light source). Note that for the dark treatment used as a control, there was no light source above the "illuminated" side. Mean values were averaged over all clones.

Fig 4. Mean movement probability between the two sides of the test boxes (±95% CI) for each clone. Black symbols denote clones with a slow life-history strategy; white symbols denote clones with a fast life-history strategy; and the grey symbol denotes the clone ME whose life-history strategy remains uncharacterised. Mean values were averaged over the two sides of the test boxes.

Fig 5. a) Predicted mean movement probability between the two sides as a function of individuals' length, for each position in the average undergrowth spectrum. White boxplots and dotted line denote that the individual was on the bright side prior to the observation, while grey boxplots and solid line denote that the individual was on the dark side. The dashed-line represent the predicted

response of the individuals that were on the bright side prior to the observation, while the solid line represent the predicted response of the individual that were on the dark side. b) Predicted movement probability between the two sides as a function of individuals' length for each clone in the maximum undergrowth spectrum.

FIGURE 1



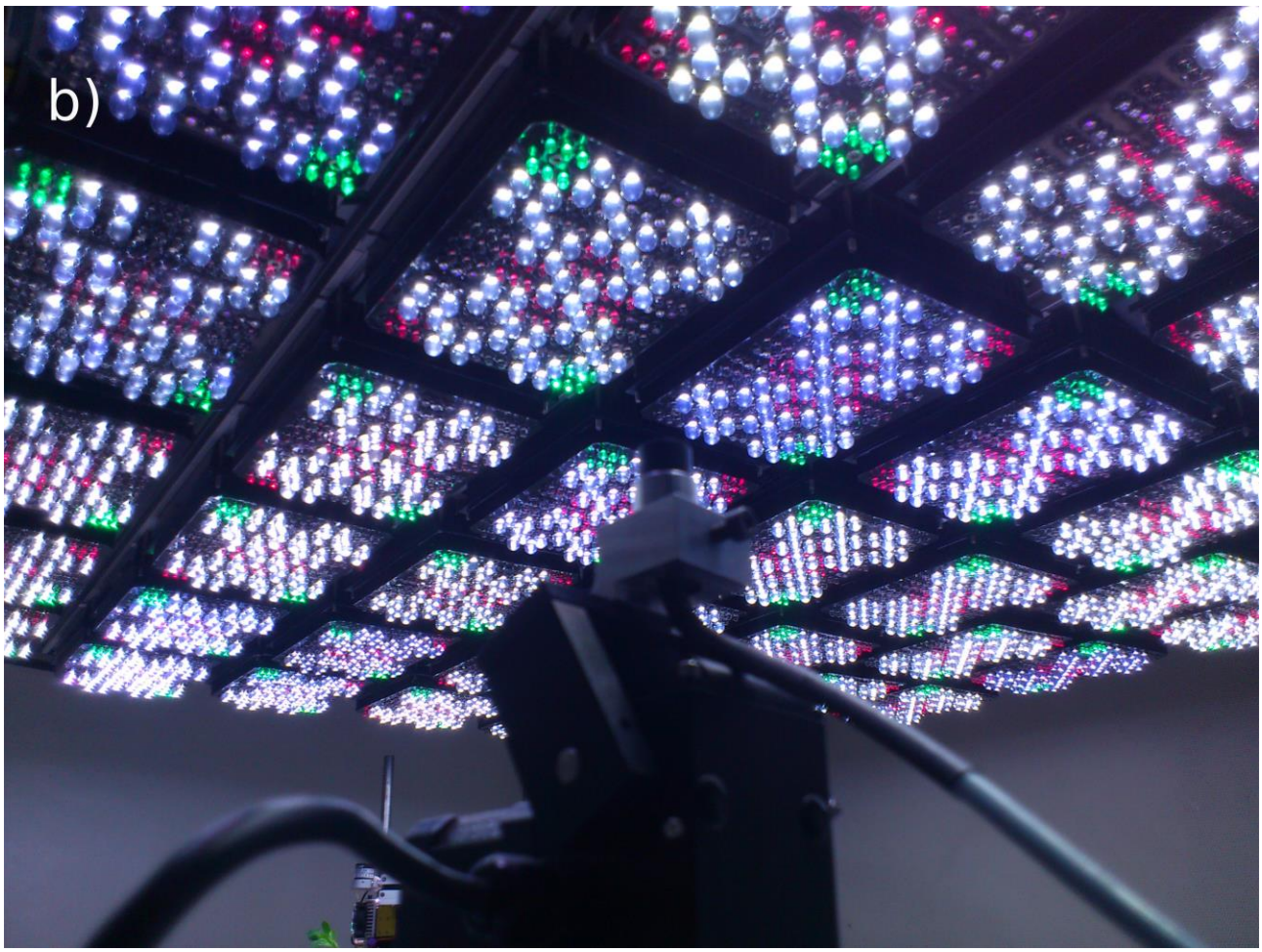


FIGURE 2

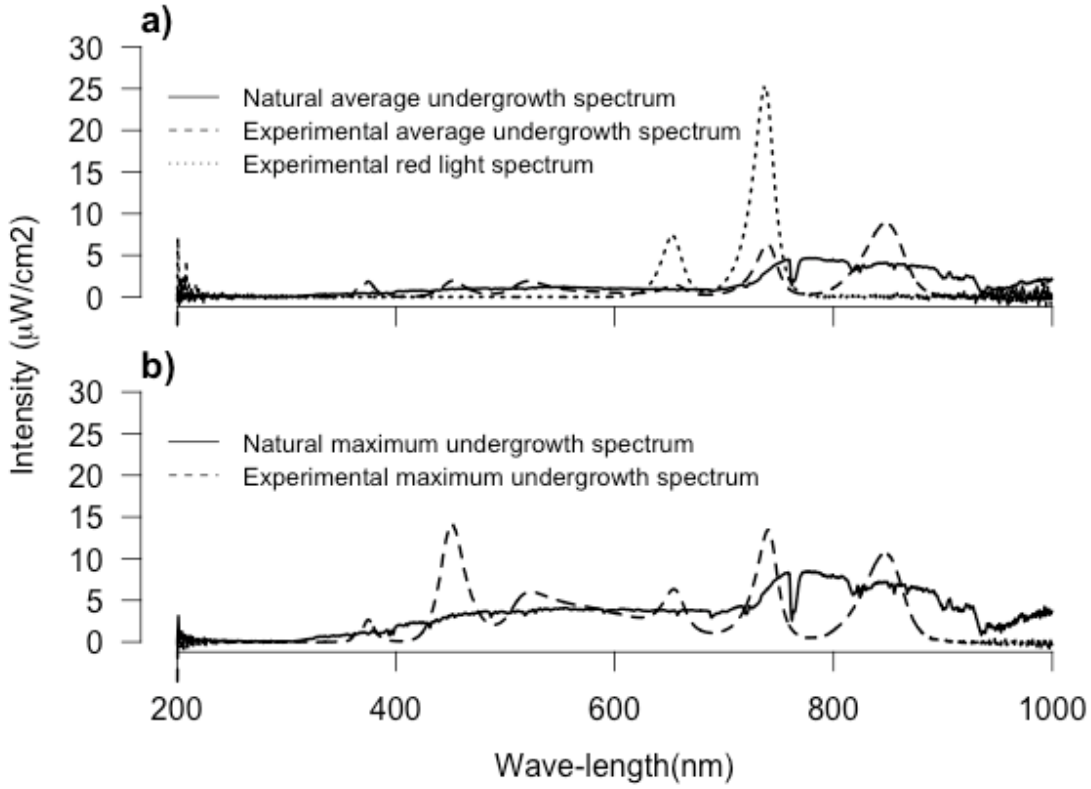


FIGURE 3

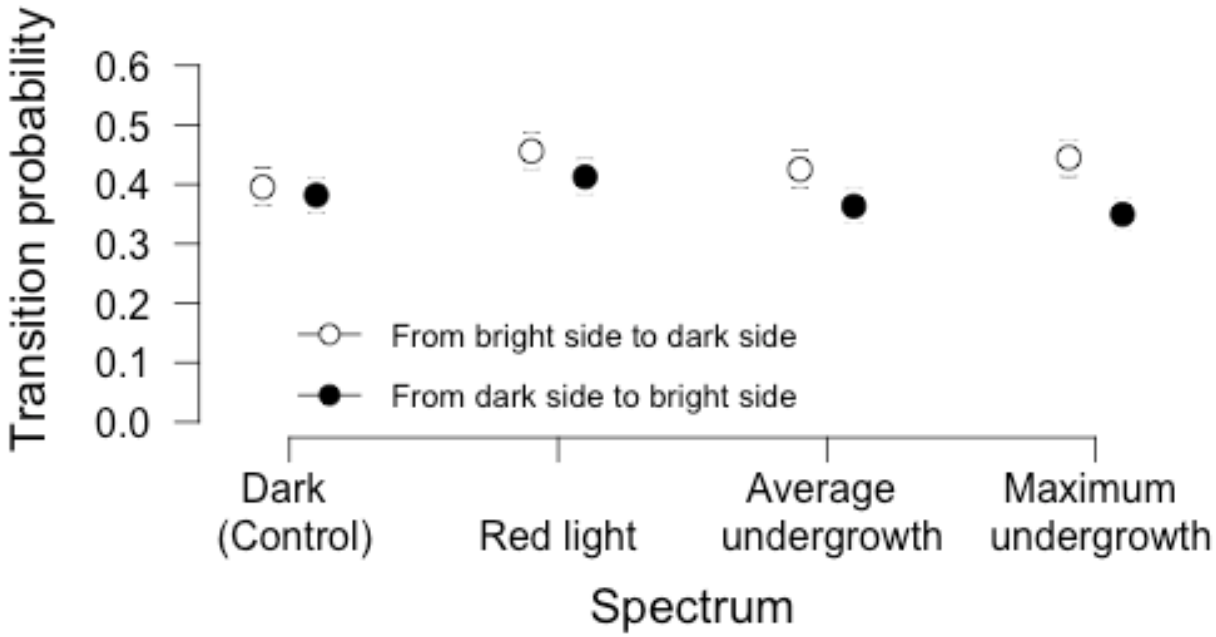


FIGURE 4

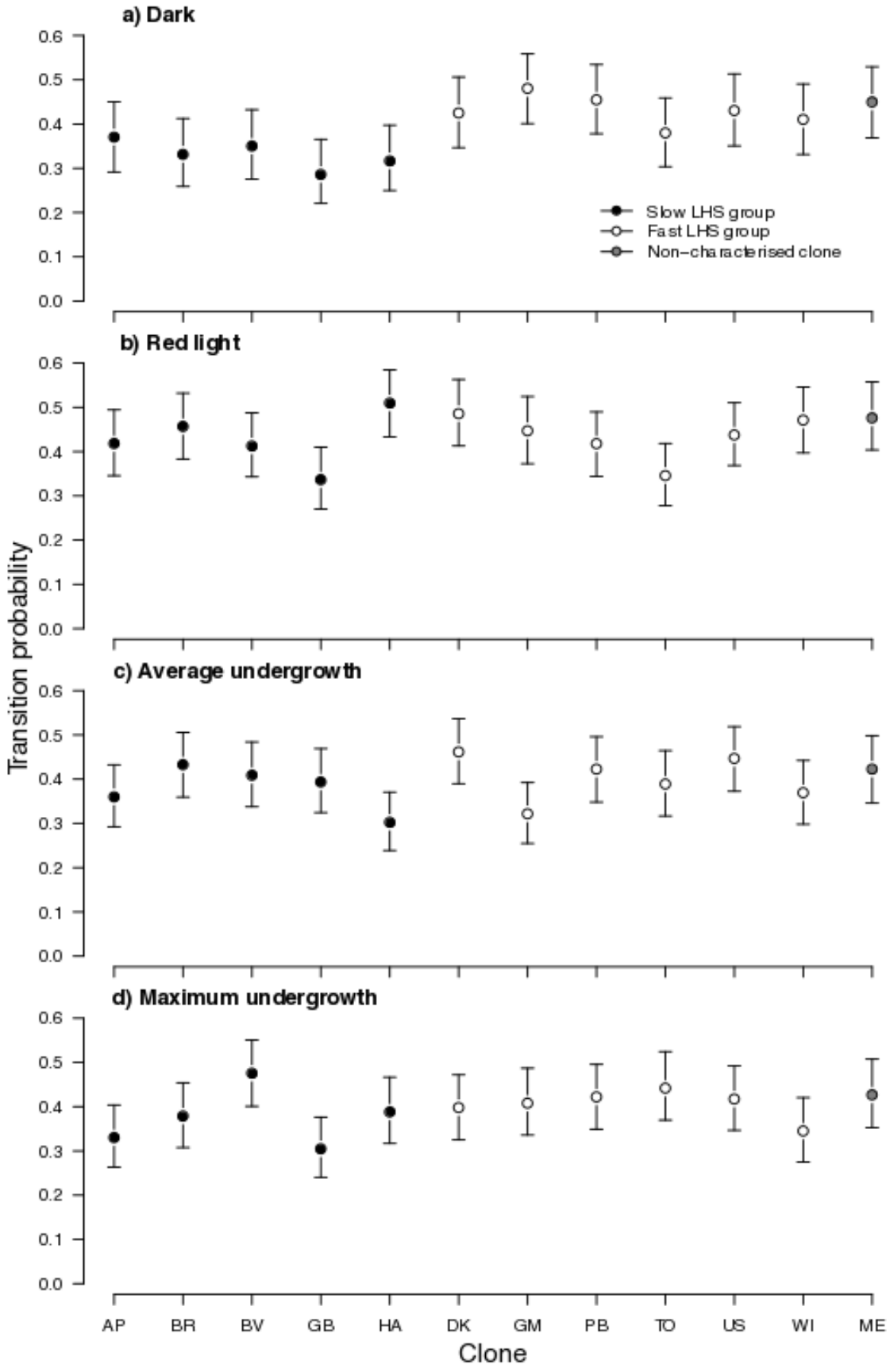


FIGURE 5

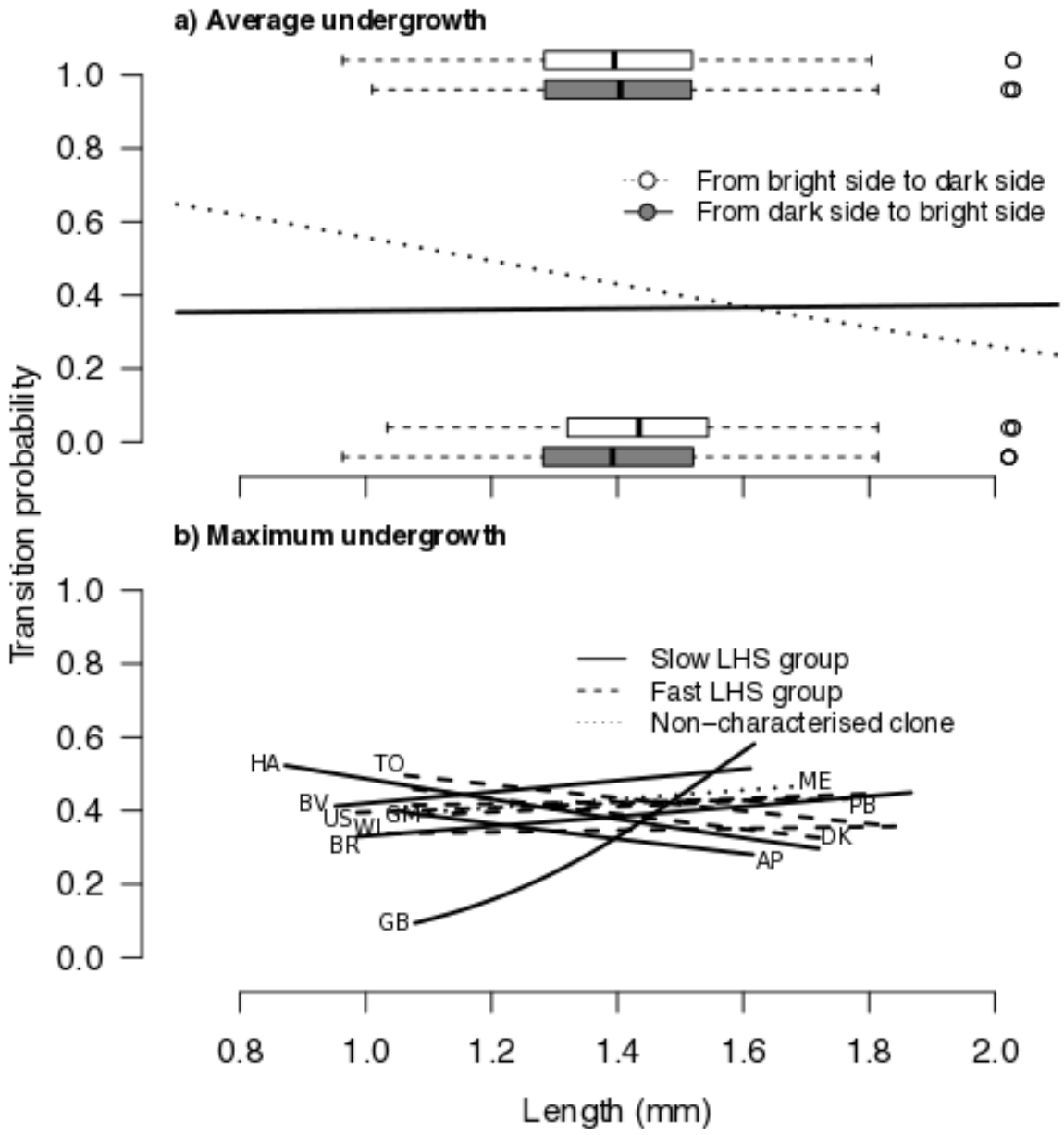


Table 1. Models that best describe transition probabilities in the dark treatment after backward selection (N=431). Dropped variables are presented in order in which they were removed. Marginal and conditional R^2 of the final model are 0.017 and 0.111 respectively.

	Variance	Std. Dev.	Chi squared	D.f.	p-value
Fixed factors:					
Clone			23.535	11	0.01485
Random factors:					
Individual	0.3471	0.5892			
Dropped variables:					
Position* Corrected Length			0.6636	1	0.4153
Clone* Corrected Length			14.338	11	0.2149
Clone* Position			17.427	11	0.0958
Corrected Length			0.0384	1	0.8446
Position			0.6487	1	0.4206

Table 2. Models that best describe transition probabilities in the red light treatment after backward selection (N=432). Marginal and conditional R^2 of the final model are 0.015 and 0.068 respectively.

	Variance	Std. Dev.	Chi squared	D.f.	p-value
Fixed factors:					
Clone			20.432	11	0.0398
Position			4.477	1	0.0344
Random factors:					
Individual	0.1895	0.4353			
Dropped variables:					
Position* Corrected Length			0.0042	1	0.9483
Clone* Corrected Length			8.9815	11	0.6236
Clone* Position			9.631	11	0.5638
Residual Length			0.0168	1	0.8970

Table 3. Models that best describe transition probabilities in the average undergrowth spectrum after backward selection (N=432). Marginal and conditional R^2 of the final model are 0.011 and 0.074 respectively.

	Variance	Std. Dev.	Chi squared	D.f.	p-value
Fixed factors:					
Position			9.6243	1	0.0019
Residual length			3.8848	1	0.0487
Position* Corrected length			6.9324	1	0.008
Random factors:					
Individual	0.2255	0.4748			
Dropped variables:					
Clone* Corrected Length			12.631	11	0.3181
Clone* Position			17.509	11	0.0937
Clone			18.088	11	0.0796

Table 4. Models that best describe transition probabilities in the maximum undergrowth spectrum after backward selection (N=432). Marginal and conditional R^2 of the final model are 0.039 and 0.089 respectively.

	Variance	Std. Dev.	Chi squared	D.f.	p-value
Fixed factors:					
Position			23.4988	1	<0.001
Clone			15.6741	11	0.1536
Corrected length			0.0312	1	0.8597
Clone* Corrected length			21.4906	11	0.0286
Random factors:					
Individual	0.1818	0.4263			
Dropped variables:					
Clone* Position			11.871	11	0.3734
Position* Corrected Length			1.2208	11	0.2692