Body-color plasticity of the English grain aphid in response to light in both laboratory and field conditions

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Abstract
The occurrence of different color patterns in a population of a species can depend on genetic variations or plasticity to environmental conditions. Body color variation is under selection because it is involved in several ecological processes such as camouflage for prey-predator interactions or resistance to environmental variations. Among insects, aphids are known to produce different body-color morphs depending on their biotic and abiotic environments and their bacterial endosymbionts. The English-grain aphid (EGA) Sitobion avenae produces both red and green morphs in cereal fields. Using both field studies on the Canadian prairies (Saskatchewan) and laboratory experiments, we aimed to study the mechanisms that trigger plasticity in body coloration to better understand the ecological role of body coloration and color-change evolved by animals, including aphids. We first analyzed green and red morph EGA distribution on wheat ears in different fields and showed that red aphids were mostly located at the top of the ear and green aphids at the bottom. Then, using DNA sequencing, we showed that red and green morphs did not strongly differ in their bacterial endosymbiont composition and abundances. Finally, using a climate-chamber setup in the laboratory, we highlighted that EGA body-coloration is under light-intensity control and that it is possible to turn aphids from green back to red within a few days, and from red back to green within a couple of weeks (low-to-high and high-to-low light intensities, respectively). Light-intensity-controlled color-change likely results in adaptive plasticity in response to shifts in environmental conditions that can occur over the lifespan of an aphid, and is fully reversible, even at the adult stage.

Keywords Behavioral ecology · Endosymbiont · Light-intensity · Plasticity · Polymorphism · Polyphenism · Sitobion avenae · Wheat

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Introduction

Many animals have evolved the capacity to produce a range of color patterns at the intraspecific level. Body color variation in the population of a species plays major roles in the animal kingdom and is involved in several ecological processes such as mate finding, thermal tolerance, food foraging, social interactions and camouflage (Duarte et al. 2017; Ford 1966; Majerus 1998; Stevens and Merilaita 2009). Body-color is thus central to evolutionary ecology because it strongly influences individual fitness in a given environment (Abram et al. 2015; Forsman et al. 2008). Color variation can be due to genetic polymorphism within a population, but can also arise from phenotypic plasticity (polyphenism), in which case body-color varies across different temporal scales depending on both biotic and abiotic environmental cues (Cloudsley-Thompson 1999; Cott 1940; Hazel 2002; Tanaka et al. 2016). Body-color polyphenism in the American grasshopper *Schistocerca americana* is, for instance, influenced by developmental temperature, with more reddish and darker forms observed at lower temperatures (Tanaka 2004). The surge damselfish *Chrysiptera leucopoma* expresses two body-color morphs that are set up during ontogeny depending on habitat background color, and that are reversible, although only before the adult stage (Fréderich et al. 2010). However, such examples of reversible color change within individuals are quite rare and unravelling the mechanistic bases of such changes is important.

Among insects, aphids (Hemiptera: Aphididae) are interesting models to study adaptive phenotypic variations because they produce different types of morphologically distinct individuals (*i.e.*, morphs) that coexist spatially and temporally (Hille Ris Lambers 1966; Dixon 1985; Loxdale and Lushai 2003; Hougardy and Mills 2008). The onset of a specific morph serves different ecological purposes and is triggered by different environmental factors. For instance winged aphids are produced to ensure dispersion in case of overcrowding on the plant (Sutherland 1969) or escaping predators (Dixon and Agarwala 1999), while sexual morphs are produced when temperature and photoperiod decrease, and lead to the production of eggs that will overwinter (Hand and Wratten 1985). Color variation has been well studied in aphids, especially in the pea aphid, *Acyrthosiphon pisum*, which displays pink and green morphs (*e.g.*, Caillaud and Losey 2010), but less in other species such as the English-grain aphid (EGA), *Sitobion avenae*, which displays a panel of colors transitioning between green and red morphs (brown, chestnut, pink, etc.) (Weber 1985; Jenkins et al. 1999). Coloration results from variation in pigment types and abundances in the tegument or the hemolymph (Bowie et al. 1966; Jenkins et al. 1999; Kayser 1982; Sullivan 2008). In *S. avenae*, apterous red morphs have a shorter longevity but a higher fecundity than green morphs, suggesting physiological differences between color morphs, but also that they could succeed differently in dissimilar environments (Araya et al. 1996).

The production of different color morphs may vary following the aphid clonal lineages which highlights the genetically-based variation of body colors (Moran and Jarvik 2010). For instance in the pea aphid in which two morphs (pink and green) are produced, color polymorphism is determined by a single biallelic locus, pink being dominant to green (Caillaud and Losey 2010). Phylogenetic analyses have recently shown that these aphid genes are derived from fungal genes (Moran and Jarvik 2010). Color polymorphism in aphids is maintained by different mechanisms, depending on the species; it is for example under balanced, density-dependent selection in the field, either by mutualistic relationships (for example with ants, Watanabe et al. 2016), or by predation and parasitism (Losey et al. 1997). Color indeed plays a major role in trophic interactions since a lot of predator insect species rely—although not exclusively—on visual cues to detect their prey, which has in...
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turn led to the evolution of camouflage strategies (Harmon et al. 1998; Thery and Gomez 2010). In the pea aphid, green morphs are more susceptible to predation by ladybugs than pink morphs which are more susceptible to parasitoids (Losey et al. 1997; Libbrecht et al. 2007). Pink morphs are more likely to drop off a plant than green morphs when attacked by a predator as a defensive behavior, but less likely to drop off the plant in the presence of parasitoids (Braendle and Weisser 2001; Dion et al. 2011). While such field-based studies have not been conducted on other aphid species, morph differences regarding sensitivity to parasitism have been reported in S. avenae. For example, the attack behavior of the aphid parasitoid Aphidius rhopalosiphi did not differ between S. avenae color morphs, nor did the aphid defense behavior, but Ankersmit et al. (1981) noted that red morphs have better physiological resistance to parasite development.

However, there can be color variation within aphid clones (i.e., among individuals sharing the same genotype) and within individuals that can be induced by plasticity to environmental factors such as temperature, photoperiod, diet, host-plant and bacterial infection (Hille Ris Lambers 1966; Markkula and Rautapää 1967; Shu-Sheng and Carver 1982; Alkhedir et al. 2010; Tsuchida et al. 2010). In this case, coloration is the result of phenotypic plasticity and may thus be reversed if the given environmental factor reverts to the previous state. For example, caterpillars of the geometrid peppered moth (Biston betularia) not only match closely the color of their host plant, but also have the potential to change color until the final larval instar if the substrate changes (Noor et al. 2008). In some aphid species, color patterns appear to be strongly linked to the presence of bacterial endosymbionts such as the obligate symbionts Buchnera aphidicola or facultative symbionts such as Hamiltonella defensa, Serratia symbiotica or Rickettsiella sp. that can provide pigment precursors that animals are unable to synthetize on their own (Douglas 1998). For instance, Rickettsiella symbionts that infect the pea aphid result in an aphid color change from pink to green (Tsuchida et al. 2010). In S. avenae, it is unknown whether endosymbiotic bacteria are involved in the body coloration process.

Having the capacity to change body-color is extremely interesting in an eco-evo perspective, however, there is still a lack of knowledge on ecological mechanisms that lead to body color-changes through plasticity in most animal species. As explained before, S. avenae does not express a strict pink-green polymorphism as A. pisum does, but rather a color gradient from green to red depending on carotenoid abundance in the hemolymph of different morphs (Alkhedir et al. 2010; Jenkins et al. 1999), which might reflect the plasticity of this trait. Eight types of carotenoids have been found in the hemolymph of brown-red morph compared to one in green morph S. avenae (Jenkins et al. 1999). Changes in light-intensity and photoperiod governs a lot of insects’ phenotypic variations across different time-scales (Saunders 2012), and might also be responsible for body-color adaptive plasticity, as reported in other animals (Lin et al. 2009; Lymbery 1992; Stegen et al. 2004; Tanaka et al. 2016). Markkula and Rautapää (1967) showed changes in the body coloration of the aphid Macrosiphum euphorbiae related to temperature, photoperiod and light-intensity, underlying seasonal changes in body coloration in the field. In some aphid species, such as the sycamore aphid, specific color may absorb solar radiation which would be advantageous in cool weather (Dixon 1972). The stink bug Podisus maculiventris can lay eggs ranging from unpigmented to heavily pigmented UV-protected eggs, which is the result of females actively controlling for egg pigmentation depending on characteristics of the laying surface (Abram et al. 2015). Other examples of adaptive responses to light exposure are known, such as Chinese longsnout catfishes that have darker skins under high light intensities, probably as a way to reduce stress (Han et al. 2005). Light-intensity thus seems to be a critical factor involved in
the plastic control of body coloration in some animal species, including aphid species. Therefore, we hypothesized that color polyphenism of EGA in the field is due to aphid exposure to different light intensities.

In this study, we first aimed to assess the levels of different morphs of aphids in the fields and their repartition on the wheat plant. We expected different color morphs to occupy different locations on the plant, either as a behavioral adaptation or as a direct response to different light intensities. Then, we wanted to understand if coloration was due to differential presence of bacterial endosymbionts. We expected bacterial communities to differ between red and green morphs, as it is already known in other aphid species. Finally, we performed lab experiments to test the effect of different light intensity exposure on EGA body-color and assessed the reversibility of this polyphenism. We expected change in light exposure to trigger plasticity in body-color of aphids, and color-change to be reversible within a generation.

Materials and methods

Field study

We surveyed five different wheat fields in the province of Saskatchewan (Canada): a commercial field near Nipawin (53.40° N, 104.56° W), University of Saskatchewan’s Kernen Research Farm (52.16° N, 106.55° W), Agriculture and Agri-Food Canada’s Melfort Research Farm (52.82° N, 104.60° W), a commercial field near Fairy Glen (53.04° N, 104.52° W) and a commercial field near Wakaw (52.63° W, 105.75° N) in summer 2015. All fields were conventionally managed. Each field was visited one time and data was collected between 10 and 26 August, when wheat ears had partially dried and harvest was underway. In each field, the first wheat plants containing at least one aphid of each morph were selected. Aphids were counted on a total of 65 wheat ears of at least 10 cm length (from 4 to 15 wheat ears depending on the fields). In most cases, both EGA morphs were found on each wheat ear when scouting the fields. Green and red morphs of *Sitobion avenae* (Fig. 1) were counted on different locations of the wheat ears using a break of 1 cm from bottom to top of the ears. First larval instar were excluded from the count because they may not get their definitive color at this point (Tsuchida et al. 2010). It is important to notice that differences in repartition patterns between morphs can only be assessed as relative proportions, because some wheat heads had more aphids of one color type than of the other.

Data was divided into “bottom” (cm 1–6) and “top” (cm 7 to top of the wheat ear) locations, which gives an almost equal repartition of the categories on the wheat ear. Morph repartition data was analyzed by fitting a Generalized Linear Mixed Model (GLMM) with Poisson distribution to the data (Bates et al. 2018) using the number of aphids as the response variable, the location on the wheat ear (bottom or top) and the morph and the interaction factor as explanatory variables, and the wheat field as a random effect. Distribution assumptions were respected and goodness of fit was visually assessed. Then, for each color morph, another GLMM was fitted to the data to assess for morph distribution differences between top and bottom. Model outputs were analyzed using the Anova function of the package ‘car’ with a Chi² statistic and Tukey Contrasts (Fox and Weisberg 2011). All analyses were carried out in R (R Core Team 2019).
CO1 barcoding

Full protocol used to confirm that both color morphs were EGA can be found as a supplementary material file to this study.

Microbial community profiling

Three adult apterous aphids of each color morph were taken from the same wheat plant from the AAFC Saskatoon Research Farm (to maximize the likelihood of obtaining differences between morphs by minimizing potential environmental or clonal variations) and total genomic DNA was extracted using a modified CTAB extraction protocol as described previously (Pérez-López et al. 2016). The genomic DNA was used as template for cpn60 universal target amplification with each reaction containing 1X PCR buffer (Invitrogen, Carlsbad, CA, USA), 2.5 mM MgCl2, 0.2 mM each dNTP, 100 nM each of H279/H280, 300 nM each of H1612/H1613 and 1U of Platinum Taq (Invitrogen, Carlsbad, CA, USA) and cycling conditions of 1 × 95 °C, 5 min; 40 × 95 °C, 30 s, 42–60 °C, 30 s, 72 °C, 30 s; 1×, 72°, 2 min. Primer sequences are listed in Supplementary Table 1. Amplicons were pooled and gel purified using Blue Pippin gel electrophoresis (Sage Biosciences, Beverly, MA, USA). Purified amplicon was prepared for sequencing using the NEBNext Universal Illumina Library Prep Kit (New England Biolabs, Ipswich, MA, USA), and sequenced asymmetrically (400 forward cycles, 100 reverse cycles) using MiSeq 500 cycle v2 chemistry (Illumina, San Diego, California, United States). Sequences were assembled into operational taxonomic units using the mPUMA pipeline (Links et al. 2013) with the relative abundance of each OTU (Operational Taxonomic Unit) determined by mapping with bowtie2 v.2.3.3.1 (Langmead and Salzberg 2012). Only full length (549–561 bp) assembled
cpn60 OTU were retained and all sequencing libraries were rarefied to the smallest library size of 1,223,121 reads prior to analysis. Bray-Curtis dissimilarity, Simpson evenness (1-D), and Shannon diversity (H’) metrics were calculated for each sample using the rarefied sequencing data. Bray-Curtis results between color morphs were tested for significance using PERMANOVA after confirming homogeneity of multivariate dispersion between groups using PERMDISP (p > 0.05). Simpson and Shannon differences between color morphs were tested using the Kruskal-Wallis test. All alpha- and beta-diversity metrics and corresponding statistical tests were calculated using the vegan package in R (Oksanen et al. 2015).

**Light-intensity experiments**

An EGA colony was created from the mixed-color morph 2015 field population, starting with approximately 50 aphids of each color morph. The aphid colony was raised on a mix of barley and wheat plants kept in a BugDorm 1 (Megaview Science, Taiwan) insect rearing cage, at 20 ± 1 °C, 16:8 h LD (Light:Dark) photo regime, and 55 ± 10% RH (Relative Humidity). Barley and wheat for the colony was grown in a growth chamber in 15-cm-diameter plastic pots containing soil-less mix (modified after Stringham 1971). Approximately 250 seeds were sown per pot which yields a pot full of many green, vegetative shoots for aphid development.

Two experiments to evaluate the effect of light intensity on aphid body-color transitions were carried out in two reach-in growth cabinets (Conviron) under two different light intensities: 13 µmol m⁻² s⁻¹ (low light intensity, T8 bulbs, with half a light bank illuminated) and 202 µmol m⁻² s⁻¹ (higher light intensity, T5 bulbs, full light bank illuminated). Both bulbs have the same color rendering properties (CRI) of 85. To provide a reference value, full sun exposure in a sunny day is 2000 µmol m⁻² s⁻¹ and 100–200 µmol m⁻² s⁻¹ would be values monitored in greenhouses with artificial lights or at the bottom of a crop canopy. Aphids are thus likely to experience a much wider light intensity range in their natural habitat, but the tested values are standard in laboratory conditions, and were sufficient to trigger plastic responses. The temperature of 20 ± 1 °C, 16:8 h LD photo regime and 55 ± 10% RH were constant and similar between chambers. For the color morph experiments, an initial three wheat (c.v Roblin) seeds were planted in a 15-cm-diameter pot and the healthiest seedling in each pot was allowed to grow while the others were removed. Experiments were started when the wheat headed and aphids were added to ears after the anthesis stage and prior to the milk stage.

**Green to red experiment**

This experiment occurred 2 months after the colony was started from field-collected individuals and the colony had been kept in the low-light chamber. Groups of ten newly moulted, apterous-adult aphids (green morph EGA) were randomly chosen from the colony and were placed on ten wheat ears (10 aphids/head, N = 100) with crispy-wrap bags tied around the stem to confine aphids to wheat ears, and put in a new growth chamber under high-light condition. Counts of each color morph were performed after 4 and 7 days by counting the number of green and red EGA adults and offspring. Counts continued until the majority of aphids had color-changed. Percentages of each color morph were calculated at each sample date by dividing the number of aphids of each color morph by the total number of aphids per ear.
Red to green experiment

We first needed to obtain red-morph aphids from our colony that only contained green-morphs, because it had been maintained 2 months under low-light conditions. To do so, one newly moulted, apterous-adult aphid (green morph EGA) was randomly chosen from the colony and placed individually onto a wheat ear (N = 10). The individual green morph EGA and their offspring rapidly shifted to predominantly (97.9 ± 0.8% mean proportion ± SD) red-morph color within 7 days when moved from low-light to higher light conditions.

Red-morph EGA were selected at the conclusion of this short process. Then, adult red morph aphids were placed on ten wheat ears (10/head, N = 100) with crispy-wrap bags tied around each ear to confine aphids, and put in a new growth chamber under low-light condition. Starting 4 days later, aphid counts were performed every 1–4 days when possible up to 63 days until the majority of the aphid population had color-changed. Percentages of each color morph were calculated at each sample date by dividing the number of aphids of each color morph by the total number of aphids per ear.

Color-morph experimental data was analysed using GLMMs with the binomial error family in R, separately for the green-to-red and the red-to-green experiments. We analysed the effect of time (days) on the combined response variable of the number of green and red aphid morphs, and we used the time (days) nested in the population identity as a random effect in the models to account for the temporal dynamic in each population (i.e., in each cage). Distribution assumptions were respected and goodness of fit was visually assessed. For the green to red experiment, we used a 2nd degree polynomial for the “day”, and for the red to green experiment, we used a 3rd degree polynomial (see the full model selection, Table S1). Model selection was operated using the dredge function from the MuMIn package, based on best-fit AIC (Barton and Barton 2019). Model outputs were analyzed using the Anova function of the package ‘car’.

Results

Field study

Overall, we found less red morph than green morph aphids in the fields (N = 3513 green EGA, and N = 1859 red EGA). There was an interaction effect of location with the type of morph on morph distribution on the wheat head (GLMM, chi² = 871.2, df = 1, p < 0.001) (Fig. 2). Red morphs EGA were mostly located at the top of the wheat ear (GLMM, chi² = 769.4, df = 1, p < 0.001; 3.96 ± 0.25 and 6.25 ± 0.32 mean ± SD aphids for bottom and top, respectively) whereas green morphs were mostly located at the bottom of the ear (GLMM, chi² = 181.1, df = 1, p < 0.001; 8.63 ± 0.40 and 3.24 ± 0.24 mean ± SE aphids for bottom and top, respectively).

CO1 barcoding

All six aphids, three from each color morph, had CO1 barcoding regions that were 100% similar to the S. avenae CO1 sequences published on the Barcode of Life Data (BOLD)
Database System confirming that the red and green aphids were color morphs of the EGA (S. avenae) and not distinct species of aphid.

**Microbial community comparison**

The microbial communities of the two aphid color morphs consisted of 136 OTU, of which 6 were likely Fungi based on BLAST comparison to the reference database cpnDB.
The sequencing libraries from all insects were dominated by OTU most closely related to *Buchnera* sp. (59–98% of sequencing reads) and *Sphingomonas* sp. (1–40% of sequencing reads) (Fig. 3a, Supplementary Table S3). The core microbiome present in the sequencing libraries for all insects was represented by bacteria from a range of phyla including Bacteroidetes, Proteobacteria, Synergistetes, Actinobacteria and Firmicutes and one fungus most similar to *Cryptococcus* sp. (77% identity).

There were no significant differences between red and green aphids with regards to community diversity or evenness (Kruskal-Wallis chi² = 1.19, p = 0.28, for both metrics) with low Shannon and Simpson 1-D values reflecting the unbalanced composition of the communities which were dominated by 2 OTUs (Fig. 3b, c). Similarly, beta-diversity analysis based on the Bray-Curtis dissimilarity metric revealed no significant differences in the composition of the microbial communities between the two color morphs (PERMANOVA, df = 1, pseudo-F = 1.18 p = 0.40) (Fig. 3D).

**Light-intensity experiments**

The proportion of green and red aphids significantly changed over time and aphids predominantly turned red within 72 h when moved from low light to high light conditions. This effect was observed in the initial population of one green-morph adult per head (97.9 ± 0.8% red at day 7) (day effect, GLM, chi² = 231.8, df = 1, p < 0.001) and the population of ten green morph EGA (80.8% ± 4.5% red by day 4) (GLMM, day effect, df = 1, p < 0.001, chi² = 254.9 and chi² = 27.7, for first and second degree polynomials, respectively) (Fig. 4). Offspring were included in population counts and the offspring of the initial adults also displayed the red-morph color. Some of the green morph EGA exhibited an intermediate yellow color before becoming red. According to model estimates, the shift from green to red in 50% of the aphids occurred around day 2.

The proportion of red and green aphids significantly changed over time (GLMM, day effect, df = 1, p < 0.001, chi² = 1060.4, chi² = 885.4 and chi² = 681.5, for first, second, and third degree polynomials, respectively). When populations of red EGA were moved from high light to low light conditions, the aphid population took weeks to fully revert from red to green morph color (Fig. 5). Some of the offspring of the red morph EGA exhibited an intermediate yellow color before becoming green. On average, there was a decrease of
0.62 red aphids per day and a corresponding increase of 0.62 green aphids per day over the course of the experiment. On average, 50% of the aphids had definitively turned from red to green by day 14.

Discussion

The sampling year (summer of 2015) marked the first year that the rarer red-morph EGA was observed (or at least, reported) in cereal crops on the Canadian prairies in recent memory (Lamb and MacKay, pers. comm.). The following growing season (2016) again had both green and red morph in the same fields (Wist, pers. obs.). We found a distribution pattern of EGA on wheat ears when we observed them in the fields in Saskatchewan, Canada. Red morphs were predominantly found at the top of the wheat head, while green morphs were mostly found at the bottom. This distribution however, does not imply that red morphs were always more abundant than green morphs at the top, but only that within each color morph, the partitioning of the color-morphs on the wheat ears was distinct. Color may thus be modified by the location of the aphid on the wheat plant and may depend on the light the aphid receives and/or on the color of the plant.

The significance of such color polyphenism in EGA can first be discussed within the context of behavioral ecology and adaptive physiology. When running behavioral experiments and placing aphids onto a new plant (not shown here), we observed that most aphids walked-back to their respective location in the plant (top and bottom) within a few hours. However, the pattern was less clear than what we observed in the field. Aphids may thus choose the location on the plant that best matches their body color. Body color in the EGA has also been reported to shift along with photoperiodic changes across the year (Jenkins et al. 1999; Markkula and Rautapää 1967). With regards to these studies and our own results, we speculate such changes may serve to synchronize the aphid coloration
to changes in plant color within seasons or to the presence of predator/parasites. Color polyphenism could be camouflage behavior to hide from parasitoids and predators since the bottom of the wheat head stays greener than the top, which turns dry and yellow first. Crypsis through background matching and/or body-color change is quite common in animals and acts as anti-predator defenses (Green et al. 2019; Merilaita and Stevens 2011; Schaefer and Stobbe 2006; Smithers et al. 2018). For example, some octopus species have mastered the art of background mimicry to escape predation (Josef et al. 2012), although using very different mechanisms from what we observe in aphids. In chameleon prawns, crypsis is maintained by adaptively changing color slowly over days or weeks to match seasonal changes in algal cover (Green et al. 2019). Crypsis behaviors that are not related to body-color are also found in insects, such as in the aphid *Eriosoma lanigerum* which covers itself with wax to escape spiders (Moss et al. 2006). Switching to a reddish color could also help *S. avenae* aphids better resist UV exposure at the top of the plants. In all organisms, melanin and other dark pigments can limit damage incurred by exposure to UV radiations, in a way that color-change by production of such pigments is highly plastic (Majerus 1998; True 2003). In aphids, Hu et al. (2013) demonstrated that the red-morph EGA exhibited stronger adaptability (e.g., better growth rate, better longevity) than the green morph at high doses of UV-B exposure. More studies are needed to confirm if this color polyphenism is an adaptive response in the field, in which case we could suspect selection on plasticity, or simply the product of environmental constraints. For example, coloration and background matching would have to be precisely quantified. It could also be interesting to perform behavioral experiments to see if the coloration and the cryptic behavior reduces parasitism or predation rates on the EGA, as it does in the pea aphid system (Dion et al. 2011; Polin et al. 2015), and to account for the great diversity of predator visual systems to test the adaptive hypothesis further (Thery and Gomez 2010).

We provided some evidence that the bacterial community within *S. avenae* aphids was very simple and dominated by only two OTUs; most closely related to the obligatory symbiont *Buchnera sp.* (89% nucleotide identity) (Oliver et al. 2014) and *Sphingomonas sp.* (89% nucleotide identity) commonly associated with phloem-feeding insects (Gallo-Franco et al. 2019). Some of the detected microorganisms, including *Cryptococcus*, have been found previously to be associated with several species of aphid (Grigorescu et al. 2018). This simple community dominated by very few OTU was reflected in very low diversity metrics with no significant differences between communities from the two color morphs. Additionally, the Bray-Curtis dissimilarity metric showed no difference in microbial community composition between red and green morph EGA in terms of the number and relative abundance of shared microorganisms. As also pointed out by Alkhedir et al. (2010), no genes in the fully sequenced genome of the obligatory symbiont *Buchnera* have been identified coding for carotenoids, nor in two facultative symbionts (Moran and Jarvik 2010). However, one can stress that it is not necessarily the most abundant bacteria that causes the coloration effect. We found no OTU closely related to *Rickettsiella sp.* symbionts in any of the two morphs. Yet, this endosymbiont is known to be responsible for body coloration of the pea aphid *A. pisum* (Tsuchida et al. 2010). Therefore, and despite the few aphid individuals that we could sequence with the Illumina platform, we argue that body color in *S. avenae* is likely not due to different endosymbiotic bacteria that make a colored pigment. However, we cannot exclude that it is due to differential levels of pigment production by a given strain under different environmental conditions, or even by genotypic interactions with the endosymbiont depending on the aphid clone. Using aposymbiotic lineages of aphids under different conditions of light-intensities would help exploring the question of morphs coloration more in depth.
We showed that light-intensity is a good candidate for explaining differential color in EGA; both red and green morph EGA could be obtained at proportions close to 90-95% when exposed to either high or low intensity lights, respectively. The observed polyphenism in the field and laboratory is most likely due to a plastic process in response to light, that makes aphid body color shift along a spectrum from green to red, and does not seem to be linked to any kind of genetic polymorphism (as it is suspected in the pea aphid (Caillaud and Losey 2010)). We thus suggest that, depending on their location on the plant, aphids receive different light intensities so they become differentially colored. As we tested for light-intensities that were lower than what aphids are likely to encounter in the field, probably the shift from low to higher values of light intensity (or reversely) is more important to trigger plasticity than the absolute value of light intensity perceived. Our findings are consistent with the results of Alkhedir et al. (2010), who showed that red color morph formation was controlled by light. How exposure to high-light intensity triggers the pigment-production cascade in insects remains to be studied. We additionally show that color can be rapidly (i.e., within the lifespan of an individual aphid) reversed from a color to another, although the switch from green to red (around 7 days) was much faster than from red to green (around 60 days). It is very likely that being able to rapidly switch from green to red is adaptive under conditions of prolonged periods of sunlight during summer, for example.

One could speculate that aphids’ “baseline” color is green and they would produce a significant quantity of carotenoids to quickly become red when exposed to high light-intensities. Carotenoid production is however likely to cost energy and other resources, as it is the case for most pigment production in animals (Roff and Fairbairn 2013; Stoehr 2006), so any advantage would have to balance the costs. Conversely, it might be physiologically constraining and take more time to eliminate carotenoids from the hemolymph and tegument, to reverse the color back from red to green (Kayser 1982; Shamim et al. 2014). It could also simply not be time-constrained, because if costly pigment production stops, then both color-morphs would hypothetically be as performant under low-light conditions. In summer, when we sampled the aphids, light intensity is higher than when closer to fall, or spring, which would favor the production of red-morph EGA, according to our light experiment results. This is in line with the observations of Chroston (1983) who mentioned that green morphs prevailed in fall or spring, and red-morphs in summer. However, we observed twice as many green aphids than red aphids on the wheat ears we sampled during the summer of 2015. Green morphs are known to produce more alates and disperse more than red morph EGAs (Ankersmit and Dijkman 1983), which could explain the prevalence of green morphs in the short summer window of the growing season in Saskatchewan.

Although we were not able to track color changes at the individual scale, our results suggest that rapid and multiple color shifts could have occurred. For example, on Fig. 5 (red-to-green shift), we observed that for some populations, most aphids turned green around day 7, then turned back to red at day 8 before turning green again progressively until the end of the experiment. This pattern could be due to either very rapid plastic response, to stochastic changes in color, or to relative change in aphid numbers in each population due to offspring production of different colors and mortality. Alkhedir et al. (2010) observed clonal differences in the capacity to change color in response to light intensity in the EGA. Our study was focused at the population level, so we do not have information for each clone. However, in proportion, most aphids changed color, as well as their offspring, when exposed to a different light intensity. This pattern may highlight poor clonal diversity in aphids from northern limits of cultivable areas, such as Saskatchewan, where populations are likely to show a pioneering effect from Northward migration each year due to harsh winters where EGA do not survive (Van Baaren et al. 2019).
To conclude, *S. avenae* is an additional example among the animal species whose body color has been observed to change and to be reversible within individuals in response to environmental conditions. Particularly, we showed the importance of light-intensity for triggering the plastic response of color-change over the lifespan of an aphid. Our study, among many others, helps highlighting the diversity of mechanisms underlying animal body-color variations, from genetic polymorphism within populations to fast plastic changes at the individual scale. Such results also show that body-color is not necessarily predetermined during early phases of ontogeny and can, in some instances, shift during the entire lifespan of an individual. Studies considering the adaptive significance of such fast plastic color changes within individuals in the field will help better understanding their evolutionary and ecological importance in animals.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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