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Does fire disturbance change insect composition and the frequency of darker phenotypes in a grassland community?

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ABSTRACT: (Does fire disturbance change insect composition and the frequency of darker phenotypes in a grassland community?). Disturbances like fire can change the composition and frequency of certain phenotypes in communities due to selection of some characteristics that would benefit from habitat change. The present study aimed to evaluate whether fire alters the composition of a grassland insect community, and also to test whether there is a higher frequency of individuals with darker phenotypes at the community level, the intra-order level (order Orthoptera), and in a species of the “Monoplatina” group (Aلتicini). Samples were taken in a burned and an unburned area of natural grassland with sweep nets and pitfall traps. Individuals were classified in morphotypes and photographed for posterior analysis of color density with the software *AxioVision*. A total of 253 individuals were collected distributed in 52 morphotypes. Morphotype compositions were significantly different between the two grassland areas. A t-test showed significant differences in the color intensity of individuals between the two areas for all taxonomic levels; the burned area showed a higher number of darker individuals. The results suggest that the increase in the frequency of darker individuals and species in the burned grassland is mainly linked to microevolutive changes. This would be a consequence of differential selection imposed by predators, since darker colors would provide higher protection in the environment darkened by fire.

Key words: melanism, phenotype selection, Chrysomelidae, environmental disturbance.

RESUMO: (Distúrbio por fogo altera a composição de insetos e a frequência de fenótipos mais escuros em uma comunidade campestre?). Distúrbios como o fogo podem alterar a composição e a frequência de certos fenótipos nas comunidades, ao selecionar determinadas características que seriam beneficiadas pela modificação de seu hábitat. O presente trabalho teve como objetivo avaliar se o fogo muda a composição de insetos em uma comunidade campestre e testar se em áreas queimadas existe uma frequência maior de indivíduos com fenótipos escuros, nos níveis de comunidade, intra-ordem (ordem Orthoptera) e em uma espécie do grupo “Monoplatina” (Aلتicini). Em uma área campestre, onde cerca de metade de sua área sofreu um evento natural de fogo, realizaram-se coletas com rede de arrasto e armadilhas de queda nas porções de áreas queimada e não queimada. Os indivíduos coletados foram separados em morfotipos e fotografados para a análise de densidade de cor através do software *AxioVision*. Foram coletados 253 indivíduos distribuídos em 52 morfotipos. A composição de morfotipos diferiu significativamente entre as duas áreas, e para os três níveis taxonômicos avaliados, através de teste t, houve diferença significativa na intensidade de cor apresentadas pelos indivíduos das duas áreas, sendo que a área queimada apresentou um maior número de organismos de cores mais escuras. Os resultados sugerem que o aumento na frequência de indivíduos e espécies mais escuras na área de campo queimado se deve principalmente a mudanças microevolutivas, as quais seriam consequência da seleção diferencial imposta por predadores, já que cores mais escuras proporcionariam uma maior proteção no ambiente cujo substrato foi escurecido pelo fogo.

Palavras-chave: melanismo, seleção de fenótipo, Chrysomelidae, distúrbio ambiental

INTRODUCTION

Environmental factors can affect organisms in many ways, including by changing or selecting individual phenotypic characteristics (Tanaka 2004). Among these characteristics, variations in color patterns has recently receiving attention (Ahnesjo & Forsman 2006, Pompozzi *et al.* 2011, Farallo & Forstner 2012, Porter & Burghardt 2012). These studies took into account the occurrence of plasticity and polymorphisms in organisms, but studies approaching the selection of phenotypes based on differential coloration caused by environmental factors are scarce.

Abiotic factors that can potentially cause phenotypic alterations in individuals include variations in temperature and humidity of natural systems, as well as some

drastic changes like fire events. Fire events are considered beneficial for some ecosystems, because they can help maintain biodiversity and, in many cases, can also disseminate species in the environment (Pompozzi *et al.* 2011). Fire events can alter the landscape structure and the biological, physical and chemical characteristics of ecosystems (Whelan 1995). These changes can favor some individuals of certain species (Hocking 1964) or even select some groups of species, which in turn could lead to alterations in community composition (Orges & Andersen 2001).

Color polymorphism is a remarkable example of phenotypic diversity and it has been observed in various taxa (Sandoval & Crespi 2008, Hoagland 1977, Sinervo *et al.* 2001, Farallo & Forstner 2012). The occurrence

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of the same population in heterogeneous substrates and habitats can facilitate selection, which can contribute for the presence of color polymorphism (Hoekstra *et al.* 2004, Byers 1990, Rosenblum 2006, Vignieri *et al.* 2010). Besides, to hide or camouflage in the environment can be important to avoid detection by predators (Gray & Mckinnon 2007). Predation is one of the most important interspecific relations that can be accessed by evaluating individual coloration, and in turn is also intimately related and affected by camouflage or aposematism (Endler 1990).

The industrial melanism of *Biston betularia* (Linnaeus 1758) is the most famous case known of colour polymorphism, and it is the classical example of microevolution described in text books. Moreover, colour polymorphisms are also described for other animal species (Guthrie 1967, Kiltie 1989). Melanic forms occur in intraspecific taxonomic levels for individuals that show darker coloration. However, after an environmental alteration, other species from the local community may also be favored. Fire events darken the substrate, indirectly changing the contrast between organisms and the vegetation (Whelan 1995). Therefore, we could expect differences in the frequency of predation events on the organisms in communities and populations, which would alter the phenotype frequency. For instance, it was noticed that darker grasshopper species and individuals of the same species showed higher frequencies in burned areas (Hocking 1964, Karpeštam *et al.* 2012).

In this study, we assessed coloration as a response factor of individuals to environmental changes to achieve two goals: (1) to evaluate whether the environmental disturbances caused by fire in a grassland area modified the composition of local insect species; and (2) to verify if this environmental disturbance increased the frequency of darker individuals and species at the intraspecific, interspecific and intra-order levels. Our two main hypotheses are that there were differences in the composition of insect communities between burned and unburned areas (possibly linked to changes in vegetation structure) and that the frequency of darker colorations were higher in the burned areas for the three taxonomic levels studied.

MATERIALS AND METHODS

Study Area

This study was carried out in the Centro de Pesquisas e Conservação da Natureza Pró-Mata (29°28'S, 50°13'W) located in southern Brazil highlands (500 – 1000 m a.s.l.). The location has 4.500 ha of protected Atlantic Forest with three vegetation physiognomies: Altitudinal Grasslands, Rain Forest, and *Araucaria* Forest (Mello 2006). Climate comprises of warm temperatures with 2,162 mm of rainfall evenly distributed throughout the year and a mean monthly temperature of 14.4 °C (Inmet 1992). The studied area is a small (*ca.* 78 ha) insular natural grassland surrounded by Rain Forest, in which half of this grassland area suffered a natural fire event in October, 2012.

Data Collection

Herbivorous insects were collected by a paired sample design in the burned grassland (BG) and in the unburned grassland (UG) areas in January 2013. A total of 20 sampling units were randomly distributed in each grassland type. Two capture methods were used in the two grassland areas: (1) 30 sweeps on the vegetation with a net in ten 20 m long transects, and (2) 10 pitfall traps placed 20 m from each other. The pitfalls were placed along a transect 10 m away from the fire line in each grassland type.

Data Analyses

The collected individuals were photographed with a color correction card (Whibal). Then, each photograph was analyzed in the AxioVision 4.7.2 software (Carl Zeiss MicroImaging Inc). Using the program, the dorsal area of each individual was selected and the total density of color was calculated (high densities = bright tones; low densities = dark tones). A correction formula was used to avoid occasional problems with illumination differences based on the color correction card (black and white scale):

$$dCi - dCp / dCb - dCp$$

where dCi = individual color density, dCp = black color density, dCb = white color density.

Paired t-tests were performed between BG and UG (significance level of 95%) for the richness and abundance of morphotypes and also for the color density showed by the individuals at three taxonomic levels: interspecific, intra-order (order Orthoptera) and intraspecific ("Monoplantina" group, Coleoptera: Chrysomelidae). The last two taxonomic groups were chosen because of their greater abundance in our samples.

A db-MANOVA (Permutational Multivariate Analysis of Variance; Anderson 2001) with 999 permutations and using the Bray-Curtis method to calculate pairwise distances was performed to check for differences in the herbivorous insect composition between the two grassland areas. All analyses were performed in PAST 2.09 software (Hammer *et al.* 2001).

RESULTS

A total of 253 individuals from the orders Coleoptera, Diptera, Hemiptera, Lepidoptera, and Orthoptera were collected and divided in 52 morphotypes.

The db-MANOVA analysis showed significant differences between the herbivorous insect compositions in BG and UG ($F=1.773$; $df=1, 18$; $p=0.041$). On the other hand, there were no differences for richness ($t=0.59$; $p=0.56$) and abundance ($t=0.12$; $p=0.91$) of morphotypes between the two grassland areas.

The individual color densities were significantly different between the insect communities in burned and unburned grassland areas ($t=-4.60$; $p=0.001$; Fig. 1A). The individuals of Orthoptera order also showed significant differences between the two grassland areas ($t=-2.37$; $p=0.023$; Fig. 1B). Additionally, the color densities

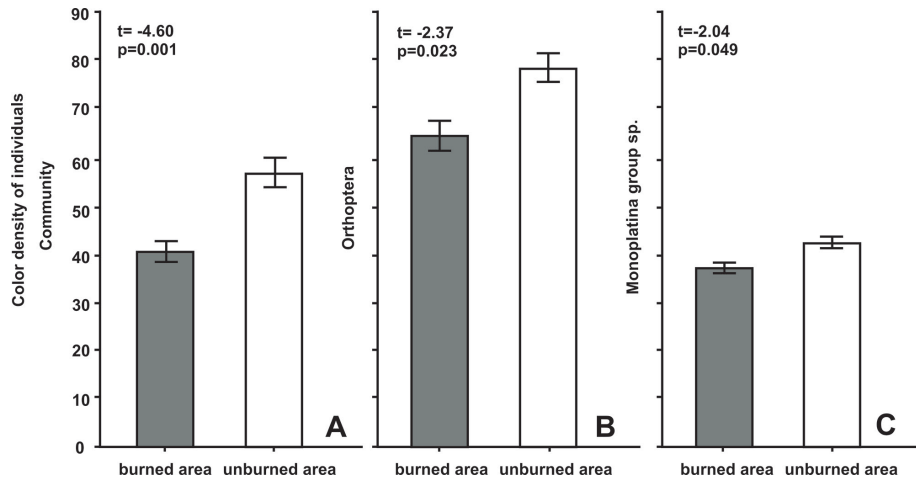


Figure 1. Color density at community level (A), order Orthoptera level (B), and “Monoplatina” group sp. (Chrysomelidae) level (C). Boxplots: filled, burned area; empty, unburned area.

of “Monoplatina” group individuals showed significant differences ($t=-2.04$; $p=0.049$; Fig. 1C). Lower intensities of color (darker tones) were more common in the burned grassland area for the three taxonomic levels (Fig. 2).

DISCUSSION

Fire events darken substrates by depositing ashes and exposing soil after vegetation removal. Fire is considered an important environmental factor driving species

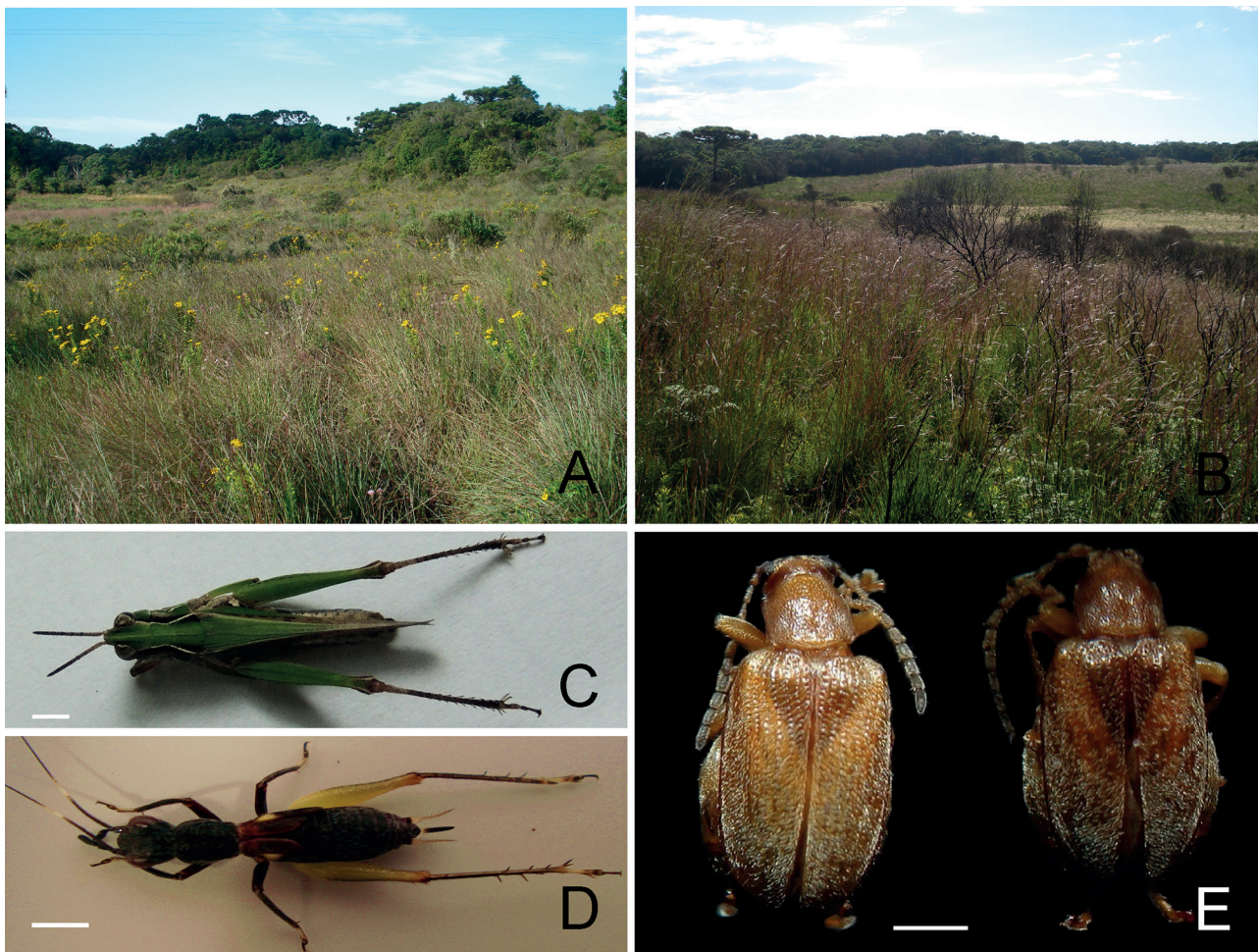


Figure 2. A. Unburned area. B. Burned area. C. Acridiidae sp. found only in the unburned area. D. Gryllidae sp., showed higher abundance in the burned area. E. “Monoplatina” group sp. (Chrysomelidae). Darker colors showed higher frequency in the burned area and lighter colors showed higher frequency in the unburned area. Scale = 1 mm.

evolution and vegetation dynamics (Bóo *et al.* 1996) by selecting species and influencing their distributions, which alters the community composition (Whelan 1995). In that sense, alterations in the physical structure of the plant community of the burned grassland may possibly explain the differences found in the arthropod community compositions. This event caused the exposition and darkening of grassland vegetation and soil, which would directly influence the distribution and occurrence of herbivorous insects (Kim & Holt 2012).

Significant differences in color density, in all assessed taxonomic levels, corroborate the hypothesis that fire indirectly affects the frequency of occurrence of light-colored individuals in the burned grassland, which would contrast with the general color of the burned area. Intraspecific fire melanism refers to the rapid increase in relative frequencies of darker color phenotypes in populations living in areas that have recently suffered this kind of disturbance (Majerus 1998). Three mechanisms have been proposed to explain possible causes of increasing proportion of melanic phenotypes: (1) natural selection, in which darker phenotypes have increased survival rates over light-colored phenotypes; (2) phenotypic plasticity, in which a darker coloration is induced in individuals due to a dark environment; and (3) dispersion and differential habitat selection, in which darker phenotypes would selectively move to areas recently altered by fire (Hocking 1964, Rowell 1971).

Caution is necessary in order to identify which mechanism is responsible for fire melanism, since in literature, it is generally characterized only at the intraspecific level and only when it is already known that the species indeed shows melanic forms in its population. However, the fact that we have found changes in the color at the community level suggests that the selection is the main mechanism acting in the increase of the frequency of darker species and individuals in the burned grassland. Karlsson *et al.* (2008) performed a long temporal study evaluating different generations of grasshoppers of a population collected *in situ* in a previously burned area and raised in laboratory. Their study indicates that the main mechanism acting in favor of darker phenotypes would be selection over plasticity or migration. In that case, the selection would act through differential predation of light-colored individuals in burned areas, because individuals possessing a color that matches with the burned habitat would be harder for predators to find (Evans & Schmidt 1990). However, this prey vulnerability is determined by complex interactions of pigments, substrate coloration, light conditions, and visual aptitude (Endler 1990), as well as by anti-predation behaviors like an appropriate habitat selection, which would reduce detection risk (Karlsson *et al.* 2008).

According to Majerus (1998), the differential predation and cryptic color selection have been considered one of the main forces behind the spatio-temporal variation in the incidence of darker color individuals, which have been found in many local populations of animal species.

For instance, it has been demonstrated that a higher frequency of melanic squirrel individuals is found in populations suffering with the incidence of frequent fire events (Guthrie 1967, Kiltie 1989). Karpeštam *et al.* (2012) investigated whether the probability of grasshopper detection would vary between different levels of fire intensity, through an experiment showing pictures to human “predators” of a grasshopper melanic form superimposed on different samples of natural habitats, representing gradual levels of fire intensity. As expected, the habitat surface darkened by fire offered a better camouflage for the grasshoppers.

CONCLUSION

As suggested by Forsman *et al.* (2011), our results indicate that the increase in the frequency of darker individuals and species in the burned grassland is mainly due to microevolutive changes, which would be a consequence of differential selection imposed by predators since darker colorations would provide higher protection on substrates darkened by fire. Dispersion and habitat selection can also be viewed as possible mechanisms for explaining our findings, however, they are unlikely because these insects are small-bodied and the directional dispersion between the two grassland areas would be difficult. On the other hand, the migration and differential habitat selection could occur as a consequence of indirect predator pressure that would force the insects to avoid predation, a pattern already described for marine copepods (Gliwicz 1986). Therefore, we suggest that future studies should use mark-recapture and behavioral observation methods in order to properly assess the selective forces influencing the patterns found in this grassland area.

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