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Color mimicry of empty seeds influences the probability of predation by birds

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Abstract. Seeds are under strong pressure from seed predators. Therefore any physical seed trait increasing the chances of the seed's survival should undergo positive selection. Seed color polymorphism, varying from pale to dark seeds, occurs in Scots pine (*Pinus sylvestris*), a keystone species of coniferous forests in Eurasia. This phenomenon can be explained by the production of large quantities of empty, always pale colored, seeds, with the opportunity for mimicry of these worthless seeds to avoid predation. Here, we investigated how the color of empty seeds may influence the foraging choices of the most common visual seed predator in temperate forests, the Chaffinch (*Fringilla coelebs*).

We show, from field experiments, that different colored seed had different probabilities of predation by the chaffinch, and that predation was highest for dark seeds and lowest for pale empty seeds. Thus, the occurrence of empty seeds might benefit full seeds which mimic their coloration, even for those which are highly visible on the ground.

In conclusion, the study demonstrated that mimicry by seeds of the color of the ground did not improve their survival but the production of pale full seeds resembling empty seeds did. This contradicts previous results and indicates that color polymorphism may reduce the predation rate by birds.

Key words: color mimicry; color polymorphism; empty seeds; optimal foraging strategy; seed predation.

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INTRODUCTION

Many abiotic and biotic factors influence seed appearance and structure. One of the most important factors influencing seed morphology is seed predation, a phenomenon where animals (e.g., granivorous birds) eat seeds and therefore strongly reduce seed survival (Janzen 1971, Hulme and Benkman 2002). Features of seeds that reduce predation are usually under strong natural selection and every feature improving survival should be favored. In the case of selection caused by predators that use visual clues, the result of the selection may be a cryptic coloration of seeds that matches the color of the background (in the tree or on the ground). This, in turn, may result in the emergence of virtually perfect background color matching by plant populations (Vignieri et al. 2010, Porter 2013, Strauss and Cacho 2013).

However, the situation where seed color matches the color of local soil is only possible either for local plant populations which have a limited possibility of gene transfer with other local populations or where a trait is under strong selection to local conditions which eliminates the effect of gene flow between populations. There are known local adaptations of cones and seed traits against different seed predators in pines (e.g., Benkman 1995, 1999, Benkman et al. 2001). The consequence of such selective pressure may be the increase of genetic isolation between local populations growing on different types of soil (Porter 2013). Due to their distinctive reproduction systems not all plants are able to adjust the color of their seeds to match the local environment.

In the case of anemophilous trees (i.e., those with windborne seeds) that are part of large and stable populations, such as the Scots pine (*Pinus sylvestris*), seed color polymorphisms occur in every population and differences between populations are either minor or do not exist (Tillman-Sutela and Kauppi 1995). In addition, an important difference compared to annual plants is that long-lived tree species produce many cohorts of seeds which can all meet distinctive selective conditions which can be caused, for instance, by varying timing of seed dispersal (Worthy et al. 2006), as well as environmental changes that influence the color of soil, for example after fires (Saracino et al. 2004, Lev-Yadun and Ne'eman 2013). Apart from environmental changes, the ideal mimicry of the soil color can be outweighed by other problems. Seeds of anemochorous species have additional structures which enable dispersal, such as wings, which significantly hamper or even make soil color mimicry unrealistic. Such structures may influence how easily these seeds are located by seed predators. For example, the existence of a wing in Jeffrey pine (*Pinus jeffreyi*) seeds causes a significant reduction of its survival chances (Vander Wall 1994).

Another possibility to avoid seed predators is mimicry where seeds resemble empty seeds that generate foraging costs without reward. Coniferous trees produce large amounts of empty seed (Kärkkäinen et al. 1999). The reason for this phenomenon is probably selection allowing for early elimination of 'bad offspring' which in turn saves energy (Kärkkäinen et al. 1999). Nevertheless, regardless of the causes, the consequence of eliminating 'bad offspring' embryos in the final phase of seed formation is the production of large numbers of empty seeds. Such Scots pine seeds are of a pale color, as in many other species of the genus *Pinus*. They are easy to differentiate from other seeds from the same tree, especially from dark full seeds. However, in the case of a seed sample from a population, visual distinction between seeds that are empty and those that contain an embryo and fully developed female gametophyte but are still pale colored is impossible. Available data indicate that the existence of empty seeds influences the probability of survival of full seeds of a given individual. This is due to the fact that they diminish feeding effectiveness and in consequence reduce seed predation (Fuentes and Schupp 1998, Perea et al. 2013). In the case of Scots pine the seeds are very well protected by the cone. Only a small number of organisms are able to feed on the seeds of Scots pine before dispersal (Worthy et al. 2006, Myczko and Benkman 2011). This situation changes drastically following dispersal, when seeds become available for many seed predators, including non-specialized bird species (Castro et al. 1999, Nystrand and Granström 2000, Worthy et al. 2006). However, in previous studies on seed predation in Scots pine the role of empty seed was ignored. In the current study we present, to the best of our knowledge for the first time, the influence of empty seeds on seed predation in Scots pine by the Chaffinch (*Fringilla coelebs*), one of the most common seed predators in temperate forests (Newton 1967).

We test the hypothesis that seeds of different colors have a different probability of predation by post-dispersal avian seed predators, because the similarity of empty seeds to pale full seeds should deter birds from foraging on the latter.

MATERIAL AND METHODS

Study area

We conducted experiments on bird foraging on Scots pine seeds in mixed forests around the city of Poznań (52°24′ N, 16°55′ E), in western Poland. In these forests Scots pine was the dominant tree species; study sites included a sparse number of oaks, birch and other deciduous species but no other coniferous species were present. The study was conducted in the springs of 2011, 2012 and 2013, i.e., during the annual peaks of natural dispersal of Scots pine seeds. We also noted the following avian seed predators in the study area: Fieldfare (*Turdus pilaris*), Wood Pigeon (*Columba palumbus*) and Greenfinch (*Chloris chloris*), but only Chaffinch was recorded foraging in the study plots.

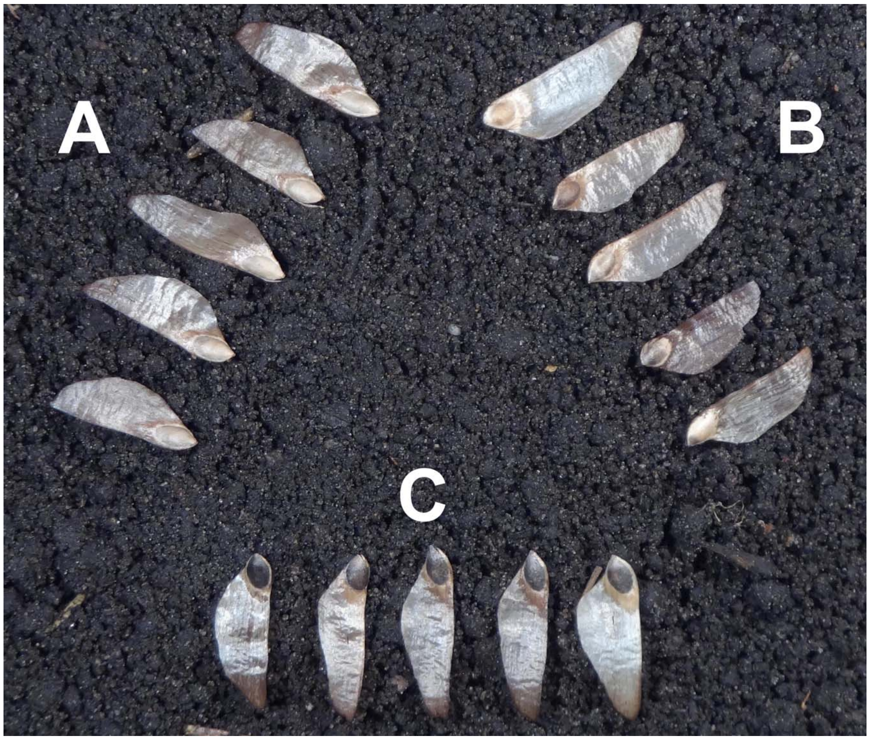

Fig. 1. Categories of seeds: (A) pale full seeds, (B) pale empty seeds, (C) dark full seeds.

Seeds

We used seeds retrieved from 120 individual Scots pines that were growing on the edges of the Zielonka forest complex (52°31′ N, 17°01′ E). Trees were established from natural regeneration and were at a low density, which allowed us to collect the cones from standing trees. The cones from each tree were collected separately and seeds where extracted in the laboratory where the color of seeds was classified. Thereafter, seeds from each tree were divided by weight into two groups: full and empty. Once the classification process was completed, its correctness was checked by cutting a sample of 30 seeds from each category and checking the stage of development of the seeds. In the case of any classification error, the seeds of that tree were eliminated from the experiments. During sorting, special attention was devoted to not damaging the seed wings, and only seeds with a developed seed coat and undamaged wing were used in the experiment. In order to avoid problems of categorization after conducting the experiment the only full seeds used in the study were of a pale or dark color, i.e., any seeds of an intermediate color were excluded from experiment. Seeds were then classified into the following categories: (1) pale full seeds, (2) dark full seeds, and (3) empty seeds (of pale color; Fig. 1). Seeds were mixed before the experiments. There was no significant difference in weight between the full seeds of pale and dark colors, which is in accordance with data gathered by other authors (Grzywacz and Rosochacka 1980, Nystrand and Granström 1997).

Experiment design

Two experiments were run, both for three years. The first was designed to closely match the proportion of seed categories in natural conditions: 20 pale full seeds, 20 empty seeds and 60 dark full seeds. These proportions occurred in the populations of Scots pine where the study plots were located. In the second experiment equal numbers (30) of seeds in each category was used. We selected study plots in mature forest stands, with a minimum of 200 m between plots. Plots were 1 × 1 m, and located in open areas between tree trunks but not directly near bushes that could hinder spotting of seeds by birds. In total, 63 plots were used in the first experiment and 26 plots in the second. Each study plot was used once during the experiment. The soil in all plots was a dark color which resembled the darker color of the Scots pine seeds used in experiments. Seed categories were thoroughly mixed and spread evenly on the plots in order to ensure that the distribution of seed categories was random. The number of seeds in each plot was 100 (first experiment) or 90 (second experiment) which approximates the average number of seeds per square meter of a mean seed crop (Koski 1991). The experiments were conducted in the mornings during the peak time of bird activity. Dry and calm days were chosen for the experiments in order to avoid movement of seeds from the plots. Next we monitored every plot for hour-long intervals. If bird foraging occurred we collected all remaining seeds from the plot together with the remains of foraging such as seed coats and wings. If bird foraging of a plot did not occur within 5 hours it was excluded from both the experiment and further analysis since this indicated a possible lack of predators at the time of observation. After completing each experiment the remaining seeds were counted in the laboratory. Between the surveys, plots were observed in order to distinguish the bird species foraging on the seeds.

Statistical analyses

We used generalized linear mixed models (GLMM) with a binomial error variance and logit-link function to test the effect of seed type on the probability of predation. If seed was eaten it was coded as 1 and uneaten seeds were coded as 0. We included plot identity and year as random factors in analyses. Paired contrasts with Šidák-corrected P values (Šidák 1967) were used to find statistically significant differences in predation rate among different categories of seed. All analyses were performed in SPSS v. 20.

RESULTS

In the first experiment we found that all three seed categories had significantly different predation probabilities (GLMM $F_{2,6297} = 572.48$, $P < 0.001$, $n = 6300$ seeds and 63 plots). The probability of predation was highest in dark seeds and lowest in pale empty seeds (Fig. 2A). The pale empty seeds also had a lower probability of predation than the pale full seeds (Fig. 2A). We also found significant differences between plots (estimate = 1.283 ± 0.253, $Z = 5.09$, $P < 0.001$) but not between years (estimate = 0.219 ± 0.327, $Z = 0.67$, $P = 0.504$).

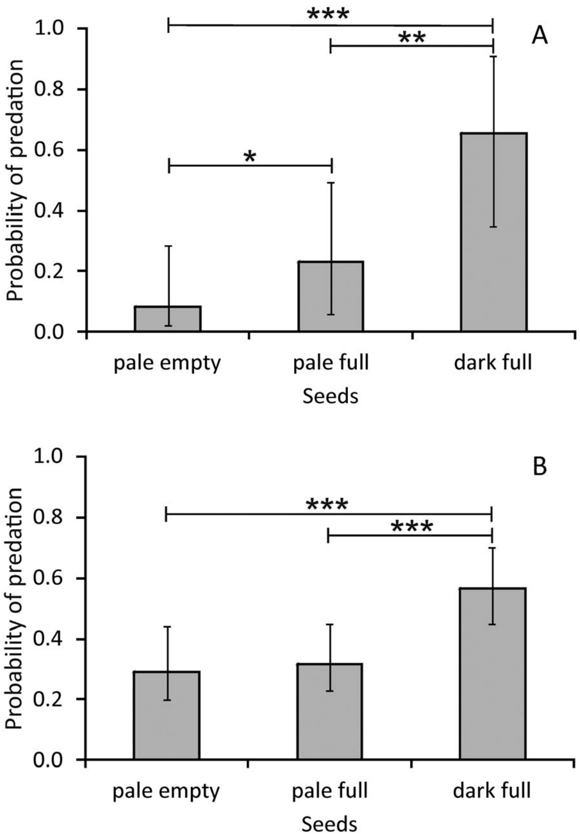

Fig. 2. Probability of predation of different seed categories in the experiments with (A) frequencies of seed resembling proportions found in natural conditions and (B) equal proportions of each seed category. Vertical bars indicate 95% confidence intervals. Statistically significant differences in probabilities are indicated by horizontal lines. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

In the second experiment we also found that seed categories had different predation probabilities (GLMM $F_{2,2337} = 69.12$, $P < 0.001$, $n = 2340$ seeds and 26 plots). The probability of predation in dark seeds was significantly higher than in both pale empty and pale full seeds (Fig. 2B). However, the predation probability of pale empty seeds did not differ significantly from pale full seeds (Fig. 2B). We also found significant differences between plots (estimate = 0.280 ± 0.095, $Z = 2.96$, $P = 0.003$) but not between years (estimate = 0.505 ± 0.930, $Z = 0.54$, $P = 0.587$).

DISCUSSION

We have demonstrated that pale full seeds may mimic empty seeds in Scots pine. For seeds with wings where empty seeds occurred, the survival of pale full seeds was significantly higher than dark seeds. Due to the presence of wings such seeds are easily detected by seed predators (Vander Wall 1994). Therefore, cryptic coloration of seeds to match that of the ground probably has little impact on the probability of survival in the period between dispersal and loss of wings since dark seeds were the most predated. In contrast, the similarity of pale full seeds to empty seeds significantly increased the chances of survival during foraging by seed predators that used visual clues (Fig. 2A). This can be explained by optimization of the foraging strategies of the birds. Previous studies have shown that the presence of empty seeds can effectively reduce the intensity of foraging on seeds before (Fuentes and Schupp 1998, Perea et al. 2013) and during dispersal (Perea et al. 2013). However, in those published cases, individual trees protected their full seeds against predation by also producing empty seeds. In our study we show that trees producing seeds of a pale color mainly benefitted from the production of empty seeds by other trees during the dispersion time. The foraging preferences of birds to dark seeds can be explained by the optimal foraging strategy to receive a constant reward, similar to that shown for abundance of food by Dark-eyed Juncos (*Junco hyemalis*) (Caraco 1981). The increase in proportions of pale full seeds and empty seeds to match those of dark seeds still maintains the protection of pale full seeds (Fig. 2B). However, in this situation, the strength of protection by mimicry by pale full seeds of empty seeds was slightly decreased.

This, of course, does not mean that the cryptic coloration of seeds to match that of the ground is not important to their chances of survival, but most likely it becomes important only after the loss of the seed wings. Nystrand and Granström (1997) showed that seed coat coloration of Scots pine matching the substrate significantly reduced the risk of predation by birds. However, in that study the authors used a commercial seed prepared for sowing, which excluded wings and empty seed. Therefore, during that experiment the choice of light-colored seed by a bird was not associated with the lack of an award, it was only energetically costly for birds in terms of the time required to find a seed. Similarly, Saracino et al. (2004) also only used full seeds when performing experiments on post dispersal seed predation on Aleppo pine (*Pinus halepensis*). In their case the cryptic coloration of seeds was most likely not the only factor affecting feeding decisions by birds because seeds that matched the color of the ground were only slightly less predated than seeds of a different color. This suggests that despite the cryptic coloration of seeds, birds are able to successfully locate them.

The commonness of Scots pine in temperate forests, with almost a continuous range in Eurasia and anemophily, most likely means that adaptation of seed color to match that of the local ground is difficult to maintain. The frequencies of seed color groups are practically stable in most of the range of Scots pine (Tillman-Sutela and Kauppi 1995) meaning that local factors probably do not play a dominant role in the maintenance of this polymorphism. Because anemophily causes the effective exchange of genes over long distances (Harju and Nikkanen 1996, Robledo-Arnuncio and Gil 2005) it makes localized adaptation of seed color to the local soil coloration insufficient to guarantee reproductive success, as in for example, the isolated populations of the legume *Acmispon wrangelianus* (Porter 2013). In addition, the very long life cycle of Scots pine means that subsequent cohorts of offspring of an individual may experience a completely different ground color. Furthermore, the frequency of predation may vary greatly during the life span of a tree. Changes in the color of the ground may occur frequently due to succession, animal activity (resulting in extraction to the surface of the mineral soil) and fires (Nystrand and Granström 1997, 2000, Svenning 2002). Therefore, mimicry of appearance to empty seed appears to be a stable alternative to mimicry of local soil coloration in a changing environment.

The differences in the color intensity in pine seeds are also correlated with the chemical composition of the seed coat. However, no association was found between the concentration of polyphenols and the frequency of seed predation (Saracino et al. 2004). The protection of seed through mimicry of empty seed may, however, generate an additional cost. Pale seeds are more susceptible to fungal infections in the early stages of germination in Scots pine (Grzywacz and Rosochacka 1980) and in an environment where there is a large accumulation of pathogenic fungi, pale seeds should be selected against. Such processes can occur especially frequently in the highly transformed nursery environment where the accumulation of pathogenic damping-off fungi is large (Le Bihan et al. 1997). Thus, the artificial replanting of Scots pine stands will be responsible for negative selection of alleles determining the bright coloration of seeds. However, this issue requires further study.

In summary, our study demonstrated that mimicry by seeds of the color of the ground did not improve their survival during dispersal but the production of pale full seeds resembling empty ones did. This contradicts previous results and indicates that color polymorphism may reduce predation by birds.

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