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# Reliable cues and signals of fruit quality are contingent on the habitat in black elder (*Sambucus nigra*)

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*Abstract*. Communication mediates interactions between organisms and can be based on signals or cues. Signals are selected for their signaling function, whereas cues evolve for reasons other than signaling. To be evolutionarily stable, communication needs to be reliable on average, but the mechanisms that enforce reliability are hotly debated in light of strong environmental influence on signals and cues. While fruit quality in black elder (*Sambucus nigra*) is unrelated to fruit color, it is indicated by alternative pedicel phenotypes. Information on fruit quality has thus been transferred from the fruit to the developmentally associated pedicels, which are environmentally determined cues. Within each phenotype, color variation indicates fruit quality. Communication by black elder is thus reliable, but the proximate mechanisms enforcing reliability are habitat specific. High irradiance increases both the contrasts of the visual cue and fruit quality in the anthocyanin-based red pedicel phenotype, while shaded plants of the chlorophyll-based green phenotype apparently use signals by forgoing photosynthesis. This is because lower chlorophyll content in green pedicels creates contrasting pedicels, and higher contrasts indicate higher sugar content in the fruits of green pedicels. Because anthocyanins are light-induced, plants use cues when exposed to high irradiance, whereas they apparently use costly signals in the shade by reducing chlorophyll content in the pedicels. In behavioral field and laboratory experiments we document that avian seed dispersers select among pedicel phenotypes that indicate different fruit quality. Plants can thus increase their reproductive success by sending highly informative cues. Our results indicate how reliable information transfer can be maintained both in cues and signals in spite of substantial environmental influence on visual traits.

*Key words: anthocyanins; black elder; communication; frugivory; handicap principle; phenotypic plasticity; plant–animal interactions;* Sambucus nigra; *sensory ecology; signal theory*.

## INTRODUCTION

Key questions in communication theory are whether communication is adaptive and which mechanisms ensure the reliability of information exchange between organisms (Searcy and Nowicki 2005). To approach these questions it is useful to distinguish whether information is encoded in cues or signals. Signals are defined as morphological, behavioral, or physiological traits that are maintained by natural selection because they convey information to other organisms, whereas cues are informative traits that evolved for reasons other than communication (Otte 1974). While cues are not necessarily reliable, some cues provide reliable information. For example, an animal's body size is a cue that may be inevitably connected to fighting ability, and thus be informative for putative opponents, but it did not evolve in response to selection by opponents (Maynard-Smith and Harper 1995).

Signals evolved to increase the fitness of the sender by manipulating the behavior of the receiver (Maynard-Smith and Harper 1995, Endler 2000). Given that the interest of organisms exchanging information often conflict, the mechanisms preventing cheating in communication based upon signals are hotly debated (Zahavi 1975, Grafen 1990, Dawkins and Guilford 1991, Lachmann et al. 2001, Cotton et al. 2004, Tibbetts and Dale 2004, Getty 2006). The handicap principle emerged as the prevailing paradigm explaining the evolutionary stability of honest signaling. It predicts that signals are reliable and informative because they represent handicaps that are costly to produce or to maintain (Zahavi 1975, Grafen 1990, Tibbetts and Dale 2004). Signal costs allow receivers to assess individual quality as they demonstrate the ability to signal and survive in spite of the handicap. Epitomized by the peacock's tail, the handicap principle became a core concept of behavioral ecology and sexual selection theory. Despite its fundamental importance, it is unknown whether the handicap principle also explains the communication in other fields. For example, there are few demonstrations of cost-enforced honest signals in the communication between mutualists (Edwards and Yu 2007).

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Pollination and seed dispersal are plant–animal interactions that are often seen as a prime example of mutualisms. As yet, it is poorly known whether communication between flowering or fruiting plants and their mutualists is mainly adaptive, that is whether it is based upon signals or whether it is primarily nonadaptive and based upon cues. On one hand, it is often assumed that variation in flower and fruit coloration reflects adaptations to divergent selective pressures of pollinators and seed dispersers, respectively. On the other hand, both flower and fruit coloration may be predominantly shaped by (from the perspective of communication) nonadaptive processes such as abiotic factors or pleiotropic relationships to vegetative traits (Traveset and Willson 1998, Armbruster 2002, Schemske and Bierzychudek 2007). As such, it is unknown whether floral and fruit colors convey information other than that a flower or fruit is present. Floral and fruit colors may convey information in the technical sense, i.e., information that reduces the uncertainty of the receiver (here the animal vector) about the quality of the reward offered by the plant (see Shannon and Weaver 1949). Currently we lack understanding of the information transfer between plants and animals and of the mechanisms that may ensure its reliability.

This is surprising as the visual traits that plants use to attract animal vectors are relatively simple and invariant as they lack behavioral components. Plant signals are thus suitable to test the reliability of information transfer as well as associated costs (Schaefer et al. 2004). While fruit color indicates fruit ripeness, ripe fruits that differ in nutritional quality often share the same color (e.g., raspberries and bitter nightshade are both red). Hence, fruit color is believed to convey limited information on nutritional fruit quality (Wheelwright and Janson 1985). The decoupling of signal and nectar reward in flowers further complicates quality assessment by pollinators (Schaefer et al. 2004). Animals' restricted ability to assess nutritional rewards might promote dishonest signaling as exemplified by the high number of deceiving orchids that do not provide any nutritive rewards (Jersáková et al. 2006).

Many plant displays consist of accessory colors in vegetative tissue in addition to fruit and flower colors. The adaptive significance of these accessory colors that are developmentally associated with reproductive organs is poorly investigated. Traditional hypotheses propose that these traits function as alerting signals of beginning ripeness and that they might increase the detectability of fruit and flower displays (Stiles 1982, Willson and Thompson 1982). Yet, these structures might also represent informative signals to animals. Pollinators selected accessory bracts as the most visible signals of nutritional rewards owing to phenotypic correlations between bract size and resin gland size in *Dalechampia* blossoms (Armbruster et al. 2005).

The simplicity of visual fruit signals provides an ideal system to examine controversial aspects of the reliability of information transfer. In particular, honest information transfer remains contentious because (1) it has been difficult to establish which signal or cue carries information as most communication systems consist of multiple signals or cues. More importantly, (2) theoretical and empirical evidence for signal costs is mixed, and it is controversial whether advertising is a monotonically increasing function of senders' quality (Dawkins and Guilford 1991, Lachmann et al. 2001, Cotton et al. 2004, Getty 2006). Finally, (3) theory predicts that environmental determination of signals may further compromise their information content (Greenfield and Rodriguez 2004). Habitat-specific phenotypes of black elder (*Sambucus nigra* L.) fruit displays present the unique opportunity to address these challenges.

Using a suite of different experiments, we tested the concept of reliable information transfer in black elder by analyzing fruit advertisement and fruit quality. To establish that seed dispersers would benefit from reliable information, we first tested the fundamental premise that the quality of fruit rewards is variable within the population. Second, we examined the relationship between fruit quality and fruit or pedicel color in vegetative tissue to test if attending to plant traits reduces the uncertainty of seed dispersers about fruit rewards. To evaluate whether communication between black elder and seed dispersers is adaptive and based upon signals, third, we discuss environmental determination of habitat-specific phenotypes. We scrutinize the basic assumption of handicap signals, i.e., that pedicel color is a monotonically increasing function of fruit quality and ask whether traits indicating quality are likely associated with costs related to signal production. We also tested the alternative hypothesis that pedicels are alerting signals of beginning ripeness. In the second part of the article, we describe experiments conducted to assess whether birds, the typical seed dispersers of black elder, select traits that reliably convey information, i.e., that are associated with higher and less variable nutritional rewards in field and laboratory conditions.

## Materials and Methods

### *Study site and tree observations*

The study was carried out in a deciduous forest near Freiburg, Germany (48° N, 7.51° E), on an area of ~50 ha. Black elder is a widespread bird-dispersed European small tree of open areas and woodland edges and one of the most important fruit resources for birds in central and northern Europe (Snow and Snow 1988, Atkinson and Atkinson 2002). This species shows phenotypic plasticity in the coloration of the pedicels of the infructescence, which redden prior to fruit ripening in sunny conditions but remain green in shady conditions of the forest interior (H. M. Schaefer, *personal observation*). Given that black elder is a characteristic plant for disturbed sites, such as gaps, woodland margins, or hedgerows (Atkinson and Atkinson 2002), we found more individuals with red pedicels ($n$ = 82) than individuals with green pedicels ($n = 40$) in the forests surrounding Freiburg. To characterize the seed dispersal system of black elder at our study site, we observed a subsample of 20 trees (10 with green pedicels and 10 with red pedicels) for a total of 162 hours. We observed fruit removal from individual plants for a total time of 8.1 ± 0.2 h (mean ± SE) from a distance of 10–20 m. Observations were carried out between 07:00 and 16:00 hours. Each plant was observed for a period of 1–2 h on a given day. Observation periods were spread out evenly to cover the entire day and to control for biases caused by differential activity at different times of the day.

For color measurements and biochemical analyses, we sampled 41 black elder trees, 22 individuals with red pedicels and 19 individuals with green pedicels. At the beginning of ripeness in early August, we counted the number of all fruits for plant individuals with a crop size of <10 000 fruits. For the remaining individuals we counted all infructescences and all fruits in 15 infructescences. We multiplied the mean number of fruits per infructescence with the number of infructescences on the entire plant to estimate the total crop size.

### *Color analysis*

For each plant, we measured the reflectance of five infructescences and 20 ripe fruits per infructescence (i.e., 100 fruits per individual) with a UBS 2000 spectrometer (Ocean Optics, Duiven, The Netherlands) and a Top Sensor System Deuterium-Halogen DH-2000 as a standardized light source (see Schaefer et al. 2007). Reflectance was measured from 300 to 700 nm as the proportion of a standard white reference tile (WS-2). The sensor was mounted inside a matt black plastic tube to exclude ambient light. We measured the reflectance of 10 black elder leaves from each plant as the background against which fruits are displayed. We also measured the reflectance of 10 leaves in 50 sympatric plant species (trees and shrubs) to account for overall background variability. Both background types yielded similar results of visual contrasts. Black elder occurred at our study site in gaps and inside the forest. We measured irradiance using a cosine-corrected probe, which collects ambient light over an angle of 180°, at 20 locations of black elders in gaps and 20 locations of black elder in the forest. The measurements were done 2 m above the ground at 09:00 hours. For these measurements, we calibrated the spectrometer with a calibration lamp of known energy output (Ocean Optics LS-1-CAL).

To calculate the conspicuousness of fruit displays according to avian vision, we multiplied the mean reflectance spectra of each structure (fruits, leaves, and infructescences) from each individual with the mean respective ambient light spectra and with the spectral sensitivities of the four avian cone types used for color vision according to an avian eye model (Vorobyev and Osorio 1998) (for calculations, see Appendix B). The visual system of birds is relatively conservative (Ödeen and Håstad 2003). The spectral sensitivities of most birds including Blackcaps (*Sylvia atricapilla*) are unknown. Similar to other passerine families, Sylviidae belong to the UVS type of color vision, where the sensitivity of the short-wavelength cone is biased toward the ultraviolet (Ödeen and Håstad 2003). Because black elder is predominantly consumed by passerine birds (Atkinson and Atkinson 2002), and at our study site exclusively by passerine birds including Blue Tits (*Cyanistes caeruleus*), we used the (UVS) spectral sensitivities of this species. However, if spectral sensitivities of the other, VS, type of avian vision are employed, the results remain unaltered (data not shown). The vision modeling enabled us to analyze the color differences between fruits, pedicels, and leaves such as they are perceived by birds (see Appendix B). We refer to these color differences as chromatic contrasts throughout the paper and use the term achromatic contrasts to refer to differences in the intensity of reflected light between two surfaces such as black fruits and green leaves. Achromatic contrasts were calculated following Schaefer et al. (2007).

### *Fruit morphology and chemistry*

After reflectance measurements, fruits were frozen, fruit size was measured before fruit were thawed, pulp and seeds were separated, and all fruit material including the juice of the watery fruits was dried for chemical analysis. Water content was analyzed as the difference between fresh and dried fruit mass. All biochemical analyses of fruit content were based on 200–300 fruits per plant individual. We used a D-glucose/D-fructose kit (Boehringer, Mannheim, Germany) for photometric analysis of sugar content because this method is unaffected by the presence of anthocyanins. For each individual we ran three replicates and used the mean value for further analyses. We used Soxhlet and Kjeldahl analyses to calculate lipid and nitrogen content, respectively. To assess protein content, we used the conversion factor of 6.25.

To evaluate whether color production might be associated with costs, we assessed pigment content in pedicels employing two widely used reflectance indices that capture >90% of variation in chlorophyll content and anthocyanin content, respectively (Gamon and Surfus 1999). The modified normalized difference vegetation index measures chlorophyll content as $(R_{750} - R_{705})/(R_{750} + R_{705})$ where $R$ is the reflectance of a given wavelength and the subscripts denote the particular wavelength. The index of anthocyanin content measures red : green ratio as the summed reflectance in the red area from 600 to 699 nm divided by the summed reflectance in the green area (500–599 nm).

### *Field experiment*

Visitation rates of seed-dispersing animals often depend on neighborhood effects (Kwit et al. 2004, Carlo 2005) and on individual crop size (Sallabanks 1993, Ortiz-Pulido and Rico-Gray 2000). Because plants with alternative phenotypes differed in habitat and crop size, we removed branches from the trees to test whether birds attend to pedicel color excluding the confounding effects of habitat and crop size.

We planted 100 black elder branches at random locations (both gaps and forest interior) in the forest to assess whether pedicel coloration functions as an alerting signal of beginning ripeness. The branches had 1 m height, and they were positioned with a minimum distance of 25 m from each other and any other fruit resource. Branches were attached to a T-shaped wooden frame to facilitate landing by avian frugivores. They were divided into an experimental group with a single infructescence of unripe fruits and either red or green pedicel colors ($n = 25$ each) and a control group ($n = 50$) with leafed twigs but no fruits. No unripe fruits were eaten during the first week. After one week, we replaced all branches of the experimental group with branches that sported a single infructescence with ripe fruits and the same color as before and replaced the 50 control branches randomly with 25 branches that had ripe fruits and green pedicels and 25 branches with ripe fruits and red pedicels. Branches with red or green pedicels did not differ in fruit numbers ($t$ test, $n = 100$, $t = 0.16$, $P > 0.82$).

If pedicels function as alerting signals indicating the start of fruit ripening (Stiles 1982), we predicted that removal rates of ripe fruits in the second week of the experiment should differ according to pedicel color in the first week of the experiment, when fruits were unripe. Specifically, we predicted higher fruit removal in branches with red pedicels the week before ripe fruits were available compared to branches that featured green pedicels or no pedicel coloration and no fruits the week preceding the experiment with ripe fruits. Under the hypothesis that red pedicels increase the detectability and/or reliability of fruit displays, we predicted that ripe fruits are more quickly removed from displays with red pedicels independent of whether or not they sported red pedicels when fruits were unripe.

Branches were placed in water buckets to prevent rapid desiccation. We placed a pot underneath each branch to control for fruits falling on the ground; missing fruits that were not found in the pots were regarded as eaten by frugivores. Every second day, we counted the remaining fruits at the branches. The experiment ended after four days, when 37% of all infructescences were detected, because fruits started to desiccate afterwards. Because we were primarily concerned with comparing the effects of alternative phenotypes, and variation in signal strength within phenotypes was twofold, we standardized contrasts by painting infructescences with Buntlack (Obi, Germany) either red or green. Artificial colors matched natural pedicel coloration (see Appendix A).

### *Fruit selection experiment*

We tested the fundamental assumption that seed dispersers evolved evolutionary or behavioral shifts toward choosing more reliable indicators of fruit quality by examining the fruit selection of 19 adult Blackcaps kept singly in cages under natural photoperiod and constant temperature. This species is one of the most important seed dispersers for black elder (Snow and Snow 1988, Eggers 2000). We controlled for the detectability of fruit displays within the cage by manipulating brown background coloration so that both pedicel phenotypes had similar chromatic contrasts against the background ($t$ test, $n = 38$, $t = 1.64$, $P > 0.11$). We analyzed birds' food choice for two consecutive days by placing one red and one green infructescence with identical fruit numbers equally accessible to birds in the cage and compared the number of fruits eaten by each bird within 15 min. Trials were conducted at the beginning of the light period, when birds were hungry and eagerly consumed fruits.

### *Statistical methods*

To test whether red and green phenotypes are associated with specific habitats, we used a $2 \times 2$ $\chi^2$ test. To compare fruit characters among red and green phenotypes (such as fruit chemistry or fruit contrasts as seen by birds), we used $t$ tests if data were normally distributed. Data on fruit chemistry were not normally distributed and therefore log-transformed to meet the assumptions of normality and of equal variances. Fruit numbers were not normally distributed, even after transformation, and therefore were analyzed with a nonparametric Wilcoxon test. We used a paired $t$ test to test whether Blackcaps in captivity selected fruits from red pedicels over those from green pedicels. To assess the equality of variance in sugar rewards offered by red and green black elder phenotypes, we employed Levene's test. We assessed the influence of pedicel color on fruit removal in the field by performing logistic regressions on the binary variable of fruit detection. Pedicel coloration (red, green) and the presence or absence of artificial pedicel colors the week prior to the experiment with ripe fruits were used as factors in the logistic regressions. We were thus able to consider the effects of each combination of presence or absence of red or green pedicels during the two stages of the experiment. We used bivariate correlations between the wavelength of pedicel peak reflectance ($\lambda_{max}$) and nutrient quality to assess the relationship between color and fruit content. To test whether the signal strength of pedicel color reliably indicates fruit quality, we used simple linear regressions with the contrasts of pedicels against the background as the independent variable and sugar content of fruits as the dependent variable. All statistical analyses were done with SPSS 15.0 (SPSS 2006).

## Results

Black elder trees had low visitation rates of frugivores with $0.53 \pm 0.22$ fruits removed per hour (mean $\pm$ SE) by either European Robins (*Erithacus rubecula*), Blackcaps, or Blackbirds (*Turdus merula*), while Tits (*Parus* spp.) consumed fruits but rarely carried seeds away from the parent plant.

### *Fruit trait variation*

Black elder displays striking bimodal variation in pedicel coloration, which are either red or green (Fig. 1). The expression of phenotypes is related to light habitats ($\chi^2 = 28.92$, $n = 41$, $P < 0.0001$); 95.5% of individuals with red pedicels (i.e., all but one individual) occurred in gaps with high irradiance, whereas individuals with green pedicels occurred in the same forest solely (i.e., 100%) under canopy cover with low irradiance. Individuals in sunny sites (gaps), which produce red pedicels, produced 4.6 times more fruits (16 305 ± 4050 fruits/plant, mean ± SE) than plants in the shade with green pedicels (3551 ± 779 fruits/plant; Wilcoxon test, $W = 5$, $P < 0.0001$). Importantly, individuals in gaps with red pedicels produced fruits with higher sugar content than individuals in the shade with green pedicels ($t$ test, $n = 41$, $t = 2.71$, $P < 0.01$; Fig. 2).

Red pedicels are more contrasting to the visual system of avian seed dispersers than green pedicels in both light environments, gaps and forest interior (both $t$ tests, $t > 9.9$, $P < 0.001$). Red pedicels increased the conspicuousness of the overall fruit displays because they had higher chromatic contrasts against the background than fruits ($t$ test, $n = 22$, $t = 3.36$, $P < 0.01$), whereas green pedicels did not increase overall detectability as they had lower chromatic contrasts against the background than fruits ($t$ test, $n = 19$, $t = 4.68$, $P < 0.001$). The achromatic contrasts of both pedicel phenotypes were lower than those of the black fruits ($t$ tests, $n = 22$ and $n = 19$, $t > 4.79$, $P < 0.001$). While pedicel color differed markedly between phenotypes, the mean brightness of black elder fruits did not differ between phenotypes ($t$ test, $n = 41$, $t = 1.47$, $P > 0.14$).

Fruit quality was highly variable among individuals as sugar content varied almost fivefold from 2.8% to 10.2% of fresh fruit mass (6.6% ± 0.3%, mean ± SE), lipid content varied fourfold from 0.15% to 0.6% of fresh fruit mass (0.3% ± 0.01%), while protein content was almost invariant (mean 0.5% ± 0.01 of fresh fruit mass). Fruit color did not correlate with fruit nutritional content (mean brightness vs. sugar, lipids, and proteins, all $r < 0.28$, $P > 0.1$), likely because low variation in black fruit color precludes that it functions as a quality-indicating signal.

### *Reliability of information transfer*

Pedicel color indicates fruit quality because there was a strong phenotypic correlation between the wavelength of pedicel peak reflectance and sugar content ($r = 0.58$, $n = 41$, $P < 0.001$). There was no difference between phenotypes in fruit traits that are not directly linked to photosynthesis such as the content of lipids, protein, water, and fruit size (all $t < 1.5$, $P > 0.18$). Importantly, there is a strong positive association between the magnitude of chromatic contrasts and sugar quality in both phenotypes (green, $F_{1,17} = 3.07$, $r^2 = 0.36$, $P < 0.01$; red, $F_{1,20} = 2.45$, $r^2 = 0.23$, $P < 0.05$; Fig. 2). Fruit quality is thus a linear function of fruit advertisement in black elder. Pedicel color is a highly informative trait as red pedicels indicate fruits with higher sugar content, they are more contrasting than green pedicels, and they are also more reliable, indicating less variance in fruit quality than green pedicels (Levene's test, df = 1, 39, $P < 0.05$).

Pigment content in pedicels indicated sugar content in fruits. Increasing anthocyanin content in red pedicels indicated increasing sugar content in fruits ($F_{1,20} = 6.05$, $r^2 = 0.23$, $P < 0.05$), whereas higher chlorophyll content in green pedicels indicated lower sugar content in fruits ($F_{1,17} = 7.92$, $r^2 = 0.31$, $P < 0.05$; Fig. 3). There was no relationship between chlorophyll content in red pedicels and sugar content in fruits ($F_{1,20} = 0.23$, $r^2 = 0.01$, $P > 0.6$) or between anthocyanin content in green pedicels and sugar content in fruits ($F_{1,17} = 0.55$, $r^2 = 0.03$, $P > 0.4$).

### *Selection by seed dispersers*

Pedicels do not function as alerting signals because removal rates of ripe fruits from randomly positioned branches within the forest are independent of the presence of red pedicels when fruits were unripe (logistic regression, $n = 100$, df = 1, Wald $\chi^2 = 0.345$, $P > 0.5$). However, pedicel coloration influenced the likelihood that fruits were removed from these branches (logistic regression, $n = 100$, df = 1, Wald $\chi^2 = 6.60$, $P = 0.01$; pedicel color, Wald $\chi^2 = 4.08$, $P < 0.05$). Red pedicels increased the likelihood of fruit consumption by two-thirds (46% vs. 28% of green infructescences were detected).

In a paired food choice experiment, captive Blackcaps selected fruits according to pedicel coloration. They initially preferred fruits from red pedicels over those from green pedicels ($\chi^2 = 4.62$, $n = 19$, $P < 0.05$) and consumed significantly more (almost twice as many) fruits from infructescences with red pedicels ($t$ test, $n = 19$, $t = 3.24$, $P < 0.001$; Fig. 4).

## Discussion

We have shown that sugar content in fruits and visual fruit advertisement in black elder are highly variable, but that seed dispersers are unable to evaluate fruit quality based upon fruit color. We show for the first time that pedicel color in fruit displays can be an informative trait that indicates fruit quality to seed dispersers. Although it remains to be seen whether pedicel coloration also signals fruit quality in other species, a similar communication system has been described for *Dalechampia* flowers where adjoining bract size, not resin gland, was the most visible long-distance signal of resin rewards (Armbruster et al. 2005). Our data are consistent with alternative red or green pedicels being environmentally determined cues. Blackcaps attend to these dichotomous cues because they prefer fruits from red pedicels, which

FIG. 1. Alternative phenotypes of pedicel coloration occur in *Sambucus nigra*. Photo taken by H. M. Schaefer.

have higher contrasts and indicate higher and less variable sugar rewards, over fruits from green pedicels. Importantly, while alternative phenotypes represent cues, color variation within green phenotypes is best thought of as a signal of fruit quality. To distinguish between signals and cues, we discuss the mechanisms that induce pedicel colors and ensure their reliability in indicating fruit quality.

*Environmental determination of pedicel color and fruit quality*

Red and green pedicels do not represent locally adapted phenotypes that maximize fruits' conspicuousness in distinct light environments because red pedicels are more contrasting in both habitats. Instead, the habitat-specific occurrence of alternative pedicel phenotypes is explicable in light of the biochemistry of plant coloration. Red colors are imparted by anthocyanins, the pigments responsible for blue, red to purplish-black colors in most plants, including black elder (Steyn et al. 2002, Deineka et al. 2005). In vegetative tissue, the light induction of genes involved in the biosynthesis of anthocyanins is well known (e.g., Mol et al. 1996, Steyn et al. 2002, Kim et al. 2003) and regarded as a biochemical model system for studying the mechanisms of photoregulation and light-stress reactions (Mancinelli 1983). Indeed, anthocyanins often function as light screens reducing the amount of light reaching the chloroplasts and thereby lowering the risk of photoinhibition at high irradiance (Feild et al. 2001, Steyn et al. 2002). At low irradiance, anthocyanin biosynthesis often reduces the rate of photosynthesis in vegetative tissue (Steyn et al. 2002, Gould 2004), potentially explaining why red pedicels very rarely occur under closed canopy. Consistent with the conjecture that anthocyanin accumulation in pedicels is induced by high irradiance in gaps, we found that individuals can rarely (~2% of individuals at study site) sport both pedicel phenotypes if they are fully shaded on one side and fully illuminated on the other side. The development of either red or green pedicel colors is thus best viewed as a cue that is mainly determined by the intensity of irradiance.

Environmental conditions determine not only pedicel phenotypes, they also translate into differential fruit

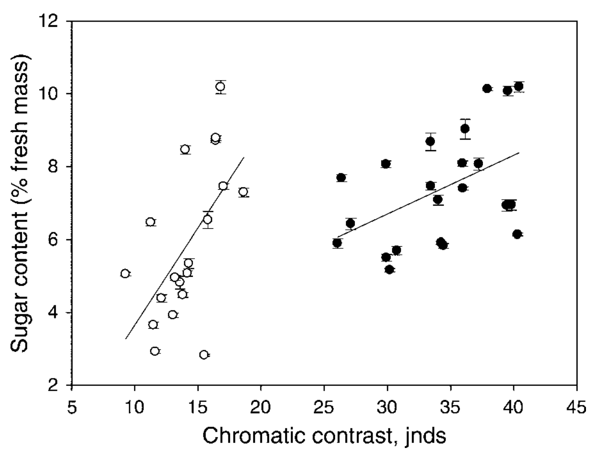


FIG. 2. Black elder fruit quality is a linear function of fruit advertisement in both alternative phenotypes. Symbols indicate mean values for plants; error bars show the standard error to depict within-individual variation in sugar content, which was measured as a percentage of fresh fruit mass. Red pedicels (solid circles) had higher sugar content and higher chromatic contrast against leaves than green pedicels (open circles). Chromatic contrasts are measured in just noticeable differences (jnds). One jnd is at the discrimination threshold for birds, values >1 jnd indicate increasing contrasts. Variance in contrasts did not differ between phenotypes (Levene's test for unequal variances, df = 1, 39, $P > 0.53$), but red pedicels had lower variance in sugar content. Although the sugar content of fruits from red and green pedicels overlaps, phenotypes differ strongly in the total amount of sugar rewards offered to seed dispersers because plants with red pedicels produce four times as many fruits.

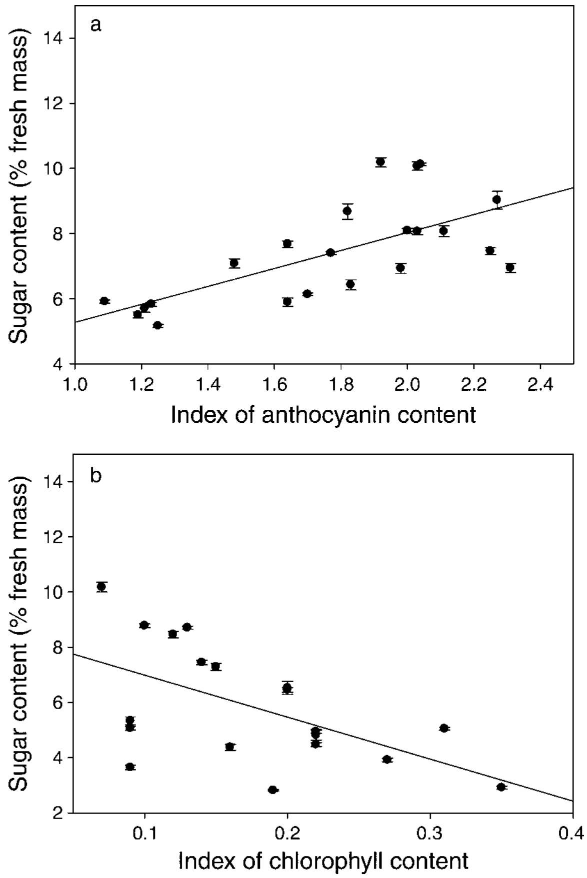


FIG. 3. (a) Anthocyanin content of red pedicels correlated positively with sugar content in fruits. (b) Chlorophyll content of green pedicels correlated negatively with sugar content in fruits. Symbols indicate mean values for plants; error bars show ±SE to depict within-individual variation in sugar content.

quality and quantity. The four times higher fruit production of plants with red pedicels is likely linked to irradiance levels as these (well known to plant breeders) often determine fecundity and fruit quality. This effect is known in black elder and apparently linked to its low shade tolerance (Atkinson and Atkinson 2002). Likewise, the sweeter fruits of plants with red pedicels are likely caused by higher irradiance in gaps that resulted in higher rates of photosynthesis and thus elevated sugar content in fruits. Note that Fig. 2 only partially reveals the overall difference in sugar investment between plants of both phenotypes as the mean sugar concentrations of plants need to be multiplied with fruit numbers, which are 4.6 times higher in plants with red pedicels. If this is done, plants with red pedicels offer by far more sugar rewards to seed dispersers than plants with green pedicels. The lower fecundity and lower fruit quality of shaded individuals with green pedicels suggests that this is an environmentally determined secondary phenotype restricted to lower-quality habitats.

*Color variation within alternative phenotypes as signals or cues*

The gradual, linear relationship between fruit quality and chromatic contrasts within both alternative pedicel phenotypes documents that the communication system between black elder and seed dispersers is more informative than class-level recognition of distinct, red or green pedicel phenotypes and their associated sugar rewards. Chromatic contrasts, i.e., the color differences between fruits and leaves in the background, are the main visual attractants of fruit displays for foraging birds (Schaefer et al. 2006, Cazetta et al. 2008) and primates (Sumner and Mollon 2000). The magnitude of chromatic contrasts of both red and green pedicels reliably indicated sugar content in the fruits. The costs that receivers pay to discriminate between signals of different chromatic contrasts within a phenotype are likely low because contrast strength is evaluated in the first neuronal steps of signal reception (see Vorobyev and Osorio 1998). Seed dispersers can thus immediately assess fruit quality by distinguishing among the contrasts created by different shades of green or red pedicels, respectively. The first important conclusion is thus that the array of informative plant traits includes various shades of green and is therefore much larger than hitherto known.

The link between variation in red pedicel coloration and sugar content in fruits is probably environmentally enforced because irradiance levels concomitantly influence the synthesis of anthocyanins and sugars. Moreover, sugars strongly up-regulate the anthocyanin biosynthetic pathway (Solfanelli et al. 2006) because the gene expression of key enzymes, such as chalcone synthase, is directly induced by sugars (Takeuchi et al. 1994). However, few individuals with green pedicels also produced fruits with high sugar content, documenting that sugar production without high irradiance does not necessarily lead to the development of red pedicels. Until

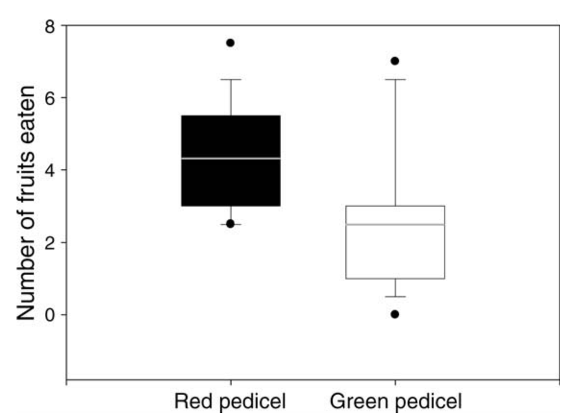


FIG. 4. Captive Blackcaps (*Sylvia atricapilla*) consumed almost twice as many fruits from infructescences with red pedicles compared to infructescence with green pedicels. Illustrated are means, inter-quartile range, 90th and 10th percentiles (whiskers), and 95th and 5th percentiles as outliers.

further evidence, the positive covariance between contrasts of anthocyanin-based red pedicels and fruit quality is probably best explicable as an environmentally determined cue.

The covariance between green pedicel colors and fruit quality is, however, not explicable as a cue. An environmentally driven cue requires that the rate of photosynthesis is positively correlated to sugar content in the fruits. However, we found the contrary: decreased chlorophyll content in pedicels indicated higher sugar content in fruits and created higher contrasts to the background. Given that lower chlorophyll content in pedicels reduces the rate of photosynthesis (Demming-Adams and Adams 1992), this result shows that sugar content in fruits is not merely a by-product of the rate of photosynthesis in pedicels. It rather suggests that black elder individuals incur costs of foregoing primary production to create more contrasting pedicels that signal high fruit quality to seed dispersers. Although the reduction in photosynthesis may seem small on the level of the entire plant, it may be particularly important for determining fruit quality as photosynthesis in the ancillary parts such as bracts and pedicels strongly contributes to initial fruit development (Whiting 1970). Finally, given that the background of leaves is optimized for photosynthesis, it is evident that contrasting signals to this background (to indicate fruit quality) can only be achieved in green tissue at the expense of deviating chlorophyll content away from the optimum. This result is consistent with increased chromatic contrasts in green pedicel colors being a costly signal, not a cue. To ascertain that green pedicels indeed function as signals, however, we need further information on how seed dispersers as signal receivers respond to color variation in these pedicels.

Two evolutionary scenarios are conceivable for the evolution of informative pedicel colors. In both pedicel phenotypes high contrasts indicating high quality fruits are associated with decreased photosynthetic activity. Such a decrease may not be costly in the high irradiance environment of red phenotypes, but it may be costly in the low light environment of green phenotypes. In the handicap scenario, the decrease in primary production in green phenotypes is per se sufficiently costly to prevent cheating by lower-quality plant individuals. Quality refers here to the resource-holding potential of plants, i.e., their photosynthetic rate. Because chromatic contrasts and fruit quality are positively correlated, sending a highly contrasting signal to the green background, which is optimized for photosynthesis, requires a significant reduction in chlorophyll content away from the optimum required for photosynthesis. Low-quality plants are possibly unable to further reduce their photosynthetic rate and produce sufficient fruits. This is consistent with the lower number of fruits produced in shaded plants and with studies showing the large influence of fruit crop on realized reproductive output (Herrera 1988). High contrasts of green pedicels would thus be handicap signals. To date, few handicap signals have been proposed in plants. Plants growing taller than physiologically required are a particularly convincing example of a possible handicap signal (Lev-Yadun 2005), while autumnal leaf coloration by anthocyanins as a handicap signal to herbivorous insects has been more contentious (Hamilton and Brown 2001, Lev-Yadun and Gould 2007). In general, handicap signals in plants involving colors are difficult to establish owing to the multiple physiological and ecological functions of plant pigments (Schaefer and Wilkinson 2004).

In the alternative scenario, signals of red and green pedicels of black elder can be cost-free, reliable, and evolutionarily stable. Reliable cost-free signaling occurs under the restricted conditions that receivers can verify each signal (Lachmann et al. 2001). In fruit signaling, the conditions are even more restrictive because seed dispersers necessarily verify each signal by consuming a fruit. Because avian seed dispersers can discriminate 1–2% difference in sugar content (Schaefer et al. 2003), they might quickly identify and shun cheating trees that advertise beyond their nutritional rewards. While we are currently unable to distinguish between both scenarios, we expect that signal verification explains why costly signals, if they occur more widely in fruits, are less exaggerated than in animal displays.

Our experiments show that Blackcaps respond to pedicel color in order to remove the more rewarding fruits. These birds thus perform class-level recognition of dichotomous cues that indicates fruit quality. In the field experiment, red pedicels might have increased the efficacy of communication because of their higher detectability as indicated by vision modeling or because they indicate higher nutritional rewards with lower variance. However, only the latter hypothesis explains why captive Blackcaps preferentially consumed fruits from red pedicels because chromatic contrasts of fruit displays in the laboratory did not differ, and adult Blackcaps do not generally prefer red over green cues when foraging for fruits (Schmidt and Schaefer 2004, Gamberale-Stille et al. 2007). Our results thus suggest that Blackcaps have evolved behavioral or evolutionary adaptations toward choosing the more reliable and more rewarding cues of fruit quality.

Based on our experiments, we predict that fruit removal rates should be higher in individuals of the red phenotype compared to that of the green phenotype. Fruit removal rates were low in the year of our study and comparable to those of less preferred species, such as woody nightshade (*Solanum dulcamara*; Snow and Snow 1988). More typically, removal rates in central Europe can be as high as 270 fruits eaten per hour (Stiebel and Bairlein 2008). Owing to the high removal success of red phenotypes that typically colonize disturbed sites, black elder may be a species that benefits from increasing light levels associated with disturbances related to human activities. In particular, the high detectability of red phenotypes and their high fruit quality might promote black elder's rapid colonization of disturbed sites such as gap or forest edges.

### *Conclusions*

We conclude that pedicels are very specific, reliable indicators of fruit quality that mediate the communication between black elder and its seed dispersers. Alternative phenotypes are environmentally determined cues, but color variation within green phenotypes more likely represents a signal produced by reduced primary production. The transfer of the quality-indicating cue from fruits to developmentally associated pedicels illustrates how indicators of quality may change over evolutionary time, which is a key process for understanding the diversity of biological signals and the maintenance of reliable communication. Although red pedicels are probably cues, their development concomitantly increases both the detectability and reliability of information exchange in black elder. Because anthocyanins are the most important pigments in flower and fruit displays, which often offer carbohydrates as main nutritional rewards, we predict that phenotypic linkage between signal design (anthocyanin production) and sugar quality of rewards are more widespread resulting in reliable plant–animal communication. Finally, we conclude that the array of plant visual signals is much larger than currently known, including various shades of green.

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## APPENDIX A

The reflectance spectra of black elder fruits, of both pedicel phenotypes, and of the artificial colors used in the field experiment (*Ecological Archives* E090-107-A1).

## APPENDIX B

The formulas used for modeling avian color vision (*Ecological Archives* E090-107-A2).