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SHORT COMMUNICATION

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Flavonoid wing pigments increase attractiveness of female common blue (*Polyommatus icarus*) butterflies to mate-searching males

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Abstract Common blue butterflies (*Polyommatus icarus*) sequester flavonoids from their larval host plants and allocate these UV-absorbing pigments to the wings. In field experiments using dummies constructed from female butterflies, mate-searching males inspected flavonoid-rich dummies more intensively than those with little or no flavonoids. Flavonoid content as signalled by UV-wing pattern may indicate ontogenetically determined female quality or enhance detectability to males.

Butterflies of a range of species in the families Papilionidae, Nymphalidae and Lycaenidae sequester flavonoids from their larval host plants (Wilson 1986a, 1987; Nijhout 1991). Among the Lycaenidae, the flavonoid content of adult butterflies in polyphagous species strongly depends on the larval host plant species actually used (Wiesen et al. 1994; Burghardt et al. 1997a; Schittko et al. 1999). Moreover, larger individuals sequester more flavonoids than smaller ones and females more than males (Burghardt et al. 1997b; Geuder et al. 1997). Hence, in species like the common blue butterfly, *Polyommatus icarus* Rottemburg, whose larvae feed on a wide variety of herbs and small shrubs in the plant family Fabaceae (Ebert and Rennwald 1991), there is ample intraspecific variation with regard to sequestration of host plant-derived flavonoid pigments.

So far, the biological significance of flavonoid sequestration for butterflies has not been resolved. Flavonoids strongly absorb UV light (Harborne 1991). Possible selective advantages discussed in the literature include protection of eggs and neonate offspring from adverse UV radiation, chemical defence against predators or microbial pathogens, or modulation of visual communication through the influence of sequestered flavonoids on wing pigmentation (Wilson 1985, 1986b, 1987).

Generally the largest share of flavonoids is allocated to the butterflies' wings. Wing colour patterns of *P. icarus* in the UV range are in fact determined by the larval diet's flavonoid content (Knüttel and Fiedler 1999). Thus intraspecific host plant-derived chemical variation of the butterflies translates into visually perceivable differences, since all lycaenid species investigated so far are sensitive to UV light (Eguchi et al. 1982; Bernard and Remington 1991; for *P. icarus*, H. Knüttel, unpublished observations). Butterfly UV wing patterns frequently play a role in inter- as well as intraspecific visual communication (Silberglied 1984; Brunton and Majerus 1995). Given the tremendous intraspecific variation of flavonoid sequestration and thus UV wing pattern in *P. icarus*, these patterns can hardly serve in species recognition (H. Knüttel and K. Fiedler, unpublished). Therefore, we set out to test whether differences in flavonoid content affect intraspecific visual communication.

P. icarus males patrol through suitable habitats in search of virgin females (Ebert and Rennwald 1991). For the more sedentary females it should be advantageous to attract mates and copulate as soon as possible after emergence to reduce their risk of pre-reproductive mortality (Zonneveld and Metz 1991). Since among the Lycaenidae the female is the flavonoid-rich sex, we specifically tested the hypothesis that flavonoid-mediated UV absorption influences the attractiveness of females to mate-searching males.

Field experiments were conducted using female dummies constructed from laboratory-bred individuals raised until pupation either on inflorescences, or on foliage, of *Medicago sativa* (see Burghardt and Fiedler 1996 for rearing method). Chemical analyses (see Schittko et al. 1999 for details on methods) revealed that flower-fed females are relatively rich in flavonoids

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(total flavonoid content, mean ± SE: 64.10 ± 5.53 μg/individual, $n = 13$, minimum: 23.49 μg/individual), whereas foliage-fed females are essentially flavonoid-free (0.20 ± 0.10 μg/individual, $n = 5$, maximum: 0.49 μg/individual; Mann-Whitney *U*-test: $z = 3.204$, $P < 0.002$). In one experimental series, dummies raised on *Trifolium repens* flowers were used (flavonoid content: 59.04 ± 7.43 μg/individual, $n = 9$, minimum: 32 μg/individual; Schittko et al. 1999).

To construct dummies, butterflies were allowed to stretch and harden their wings upon emergence from the pupa and were then deep-frozen at –20 °C. Later, butterflies were pinned, set on a special spreading board, with their wings half open at an angle of 90°, and dried at 40–50 °C for 2–3 days. Dummies thus mimicked the typical posture that female *P. icarus* exhibit while sitting in the vegetation or nectaring on flowers (Fig. 1). In field tests, free-flying *P. icarus* males significantly preferred dummies with wings half open over those with closed or fully opened wings (frequencies of attraction, half open : fully open : closed = 18 : 5 : 2, $\chi^2_{1\mathrm{df}}$ (half open vs. open, null hypothesis of equal distribution) = 7.35; $\chi^2_{1\mathrm{df}}$ (half open vs. closed) = 12.8, both significant at $P < 0.01$ using Hochberg's (1988) correction for multiple dependent tests).

Preliminary tests also showed that males prefer large females (forewing length >16 mm) over small ones (forewing length <12 mm; frequency of attraction, large vs. small = 24 : 8, $\chi^2_{1\mathrm{df}} = 8.00$, $P < 0.005$). Therefore, we selected only medium-sized individuals from mass rearings as dummies (mean forewing length ± SE, experiments in 1995: flavonoid-rich 14.10 ± 0.16 mm, flavonoid-free 14.20 ± 0.25 mm; *t*-test: $t_{20\mathrm{df}} = 0.457$, $P > 0.65$; experiments in 1998: flavonoid-rich 13.21 ± 0.32 mm, flavonoid-free 13.56 ± 0.34 mm; $t_{12\mathrm{df}} = 0.732$, $P > 0.47$).

For the actual field experiments, four dummies (two flavonoid-rich, two almost flavonoid-free) were pinned onto large, prominent patches (diameter 30–50 cm) of *Lotus corniculatus* (a major host plant of *P. icarus*: Ebert and Rennwald 1991) at a height of 15–20 cm above ground. In 1998, dummies were pinned on wooden sticks (30 cm high) surrounding a *L. corniculatus* patch and each garnished with 4–5 *L. corniculatus* flowers.

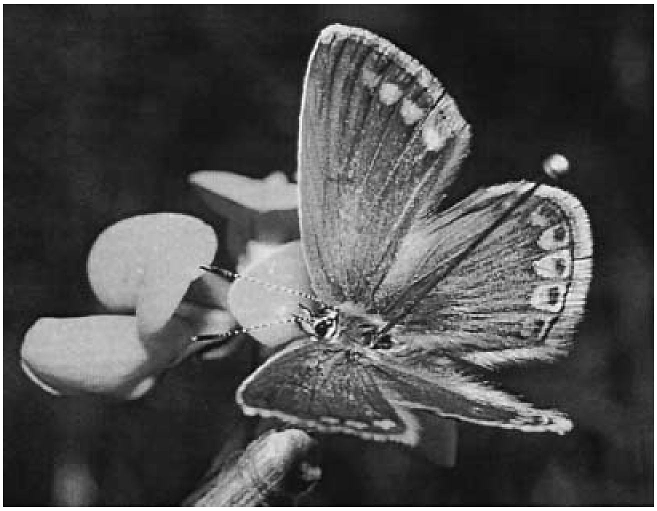

Fig. 1 Female *Polyommatus icarus* dummy pinned to *Lotus corniculatus* flower

The four dummies were arranged at the corners of a quadrat (side length 30–40 cm) in such a way that dummies on diagonally opposing corners were of equal type with respect to flavonoid content, whereas neighbouring dummies contrasted with each other. To reduce positional bias, dummies were regularly exchanged between the four positions. In each trial the forewing length of dummies differed by less than 1 mm.

Experiments were conducted between August 5 and 18 during the flight period of the summer brood of *P. icarus* in two natural populations in three years. The field sites were nutrient-poor meadows near the campus of Würzburg University (1995, 1996) and on the University of Regensburg campus (1998). We performed experiments only during periods of favourable weather (air temperature 21–35 °C) with sufficient flight activity of mate-searching males between 1100 and 1800 hours CEST. Each session lasted 1 h (or less, if weather conditions deteriorated), and then the dummies were offered at a new place. The same set of dummies was exposed in not more than 2–3 consecutive experimental sessions. In all, we assembled data during 43 h spread over 23 days, using over 65 individual dummies.

Three types of male reactions towards female dummies were clearly distinguishable. Males passing a dummy at a distance of 15 cm or less, who did not show any noticeable response, were scored as 'pass by'. If a male changed his flight path and approached the female dummy, but immediately left her again, this was scored as 'approach'. Males who circled around the dummy for one or more seconds and sometimes even alighted were scored as 'inspect'.

Results from 1132 observations of male *P. icarus* are summarized in Fig. 2, separated into experiments made under full sunshine and under cloud cover, respectively. For statistical evaluation, observations of each year were used to first calculate a χ^2 value, and significance was assessed for the sum of these χ^2 figures with the number of experimental series (i.e. years) as degrees of freedom (Sachs 1992). Overall, with both types of dummies, about 69.7% of males within a radius of 15 cm showed a noticeable response, while 30.3% of the males ignored the dummy. Irrespective of weather conditions, this fraction of by-passing males did not differ between flavonoid-rich and flavonoid-free females (sunshine: $\chi^2_{3\mathrm{df}} = 2.87$, $P = 0.41$; cloud cover (data for two years only): $\chi^2_{2\mathrm{df}} = 3.99$, $P = 0.14$). Hence, sequestration of flavonoids did not assist in attracting a larger fraction of males. There was, however, a very clear difference between both dummy types in the reaction of responsive males, if presented under bright sunshine

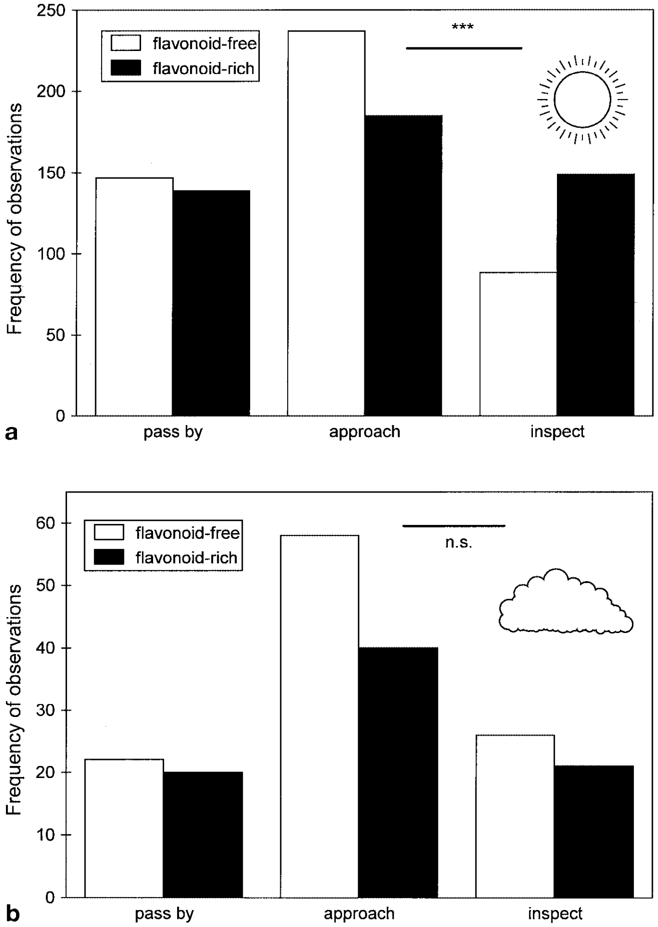

Fig. 2 Response of male *P. icarus* towards dummies made from female butterflies (*empty bars* flavonoid-free, *black bars* flavonoid-rich). '*Pass by*': male passes by a dummy at a distance <15 cm, but shows no response; '*approach*': male changes flight path towards dummy, but immediately leaves it; '*inspect*': male closely approaches dummy and circles around for at least 1 s. **a** Sunny weather, significant difference between 'approach' and 'inspect' (2×2 contingency table, ***: $P<0.001$); **b** overcast, sun covered by clouds, no significant difference (n.s.)

($\chi^2_{3\mathrm{df}}=20.69$, $P=0.00012$, significant after Bonferroni-Hochberg correction for four dependent tests on the same data-set). While at flavonoid-rich dummies, 44.6% of all responses to dummies resulted in close inspection, this proportion was only 27.1% at flavonoid-free dummies. Remarkably, under cloud cover this differential response of males no longer occurred ($\chi^2_{2\mathrm{df}}=0.87$, $P=0.64$), and only 32.4% of responses resulted in more intensive attraction. Extrapolated to living females, this implies that under bright sunshine (the favoured conditons for flight activity) a flavonoid-rich individual has a greater likelihood of causing a potential mate to closely inspect her and to initiate courtship.

Although highly significant, the extent of the difference in male reactions towards both dummy types was not very large. It must, however, be kept in mind that the intensity and spectral composition (e.g. UV fraction) of available light varies strongly with time of day, cloud cover or vegetation. This will influence the visual appearance of female dummies to searching males (Endler 1990, 1993). In fact, under overcast conditions, *P. icarus* males no longer discriminated between dummy types according to their flavonoid content, suggesting that the actual light environment plays a crucial role in visual mate choice. Moreover, motionless dummies in the natural habitat had to compete with living females. Finally, the density of the male population also fluctuated markedly from day to day as well as between years. Despite these many variables beyond experimental control, the differential response of males in our field experiments was remarkably robust over 3 years and in two different habitats.

The higher attractiveness of flavonoid-rich, strongly UV-absorbing *P. icarus* females to mate-searching males raises the question as to what information female wing pattern actually conveys. First, UV absorption may increase colour-saturation (Lunau 1992) and thus conspicuousness to searching males. Second, *P. icarus* individuals grown on inflorescences are generally richer in flavonoids (i.e. more UV-absorbing) than those nourished with foliage from the same plant species (Burghardt et al. 1997b; F. Burghardt, unpublished data). Larvae of *P. icarus* also grow faster and larger when feeding on flowers rather than foliage (Burghardt and Fiedler 1996). Therefore, the sequestration of flavonoids may, under natural conditions, signal that a female butterfly has developed on a nutrient-rich host plant, which in turn could be related to her 'quality' as a potential mate. For example, flower-fed individuals should be expected to have greater nitrogen reserves due to their protein-rich diet (Wheeler 1996) and to have greater fecundity (Honek 1993). It remains to be addressed whether flavonoid content in fact correlates with such measures of reproductive 'quality'.

Females of *P. icarus* and other lycaenid butterfly species typically sequester larger amounts of flavonoids than conspecific males. Together with the results reported here, all the evidence now points towards a role of sequestered flavonoids in intraspecific visual communication. While in brightly UV-reflecting male Pieridae it remains to be tested whether intraspecific variation is more important in signalling male 'quality' or in male–male combat (Brunton and Majerus 1995), among polyommatine lycaenids host plant-mediated intraspecific variation of UV absorption influences a female's likelihood of being courted by males. Further experiments will be needed to clarify whether this effect is really equivalent to signalling 'quality' or, alternatively, enables males to more easily locate and identify potential mates.

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