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Fluorescence in *Asellus aquaticus* (Isopoda: Asellota): a first approach

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ABSTRACT

In the freshwater isopod, *Asellus aquaticus* (Isopoda: Asellota), fluorescing metabolic products, stored in specialized cells, cause intraspecific variation in individual visibility. In many populations, 50–80% of isopods exhibit increased visibility under natural light conditions, which increases predation risk to these individuals. Furthermore, fluorescing isopods exhibit different behaviour with respect to sheltering. These individuals would be expected to be outcompeted by their non-fluorescing conspecifics. However, assortative mating of fluorescing versus non-fluorescing isopods warrants reproduction in both phenotypes. We hypothesize possible causes of the isopods' fluorescing appearance and present results that allow the predicted consequences to individual isopods to be tested.

Keywords: assortative mating, behavioural change, colour change, endoparasites, honest signal, metabolite storage, parasite-induced changes, predation risk.

INTRODUCTION

In the freshwater isopod, *Asellus aquaticus* (Isopoda: Asellota), 50–80% of individuals from different sites in the vicinity of Kiel, Germany, display yellowish dorsal stripes along both sides of their posterior body (Fig. 1a) that have not previously been described. In addition to being conspicuous under natural light, these stripes exhibit strong fluorescence of green to yellow light (550 nm $< \lambda <$ 750 nm) when observed under UV (300 nm $< \lambda <$ 400 nm) (Fig. 1b). Fluorescing yellow stripes on the back of a brownish animal crawling on dark ground enhance its visibility in shaded freshwater (authors' observations under a natural light regime) and may make it more prone to predation (cf. Bakker *et al.*, 1997). The observed high proportion of fluorescing individuals in natural populations is, therefore, surprising. Our aim here is to explain the observed intraspecific variation in coloration by discussing possible causes and by hypothesizing consequences that can be tested experimentally:

1. Fluorescence is caused, through some unknown mechanism, by parasitic endosymbionts that need to be ingested by their final hosts together with isopods that serve as prey. In

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this case, fluorescing isopods should be preyed upon at higher rates. Furthermore, non-fluorescing isopods may be expected to be preferred as mates compared with fluorescing individuals.

2. Fluorescence serves as an honest signal for mate quality with respect to resistance to high predation pressure (see 1). In this case, we would expect fluorescing isopods to have higher mating success.

MATERIALS AND METHODS

Asellus aquaticus was collected in ponds and slow-flowing creeks in the vicinity of Kiel, Germany, during the summer and early fall of 2000. Individuals were sexed, checked visually for the appearance of yellowish stripes under natural light conditions and subsequently used for fluorimetric measurements or for behavioural experiments. To characterize fluorescence of individual isopods exhibiting yellow stripes, whole-body homogenates (phosphate buffer, pH 6.5) were screened for the wavelength emitted when excited at different wavelengths (300 nm, 350 nm, 400 nm, . . . , 700 nm) ($n = 5$). For comparison, isopods without conspicuous stripes were also screened for fluorescence.

To examine changes in visual appearance in response to the light regime or temperature, we monitored seven males and seven females every 4 h for 5 days (14 h light/10 h dark, light intensity gradually increasing and decreasing; 15°C during the day, 7°C during the night).

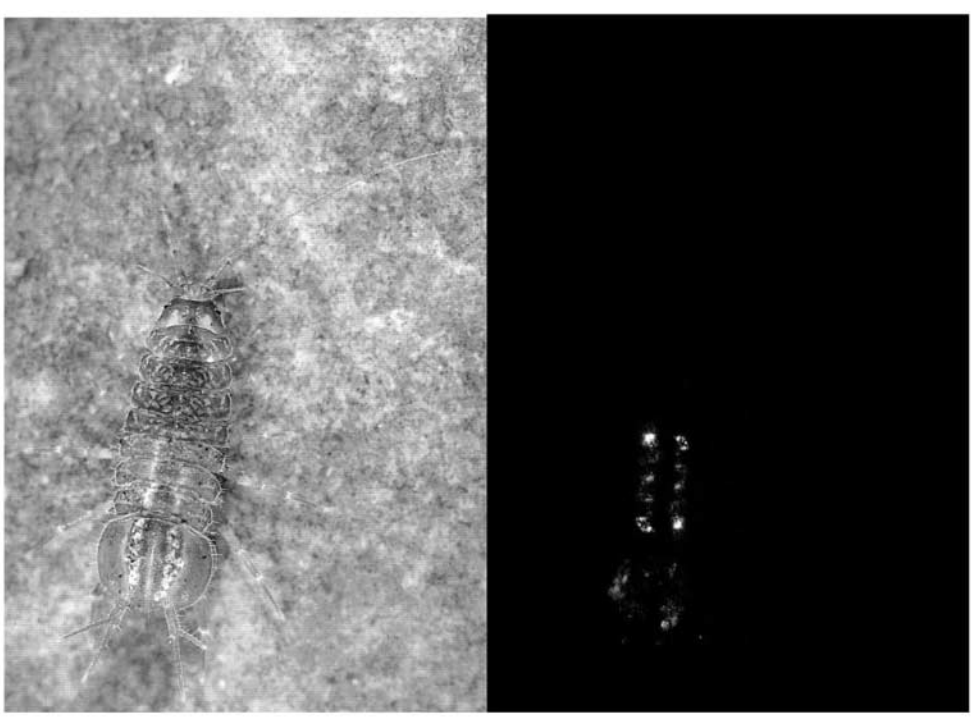

Fig. 1. *Asellus aquaticus* with yellowish Zenker cells visible under a natural light regime (left) and exhibiting fluorescence (550 nm < λ < 750 nm) when excited with UV (300 nm < λ < 400 nm) (right).

Another set of seven males and seven females was checked every 3–4 days for 3 months to detect long-term changes associated with moulting.

Feeding experiments were performed as described by Bakker *et al.* (1997) using three-spined sticklebacks (*Gasterosteus aculeatus*) as predators and fluorescing and non-fluorescing isopods as potential prey ($n = 30$ each). Furthermore, individual fluorescing and non-fluorescing isopods ($n = 15$ each) in translucent plastic containers were observed for sheltering behaviour for 10 min. Behaviour was categorized as 'resting beneath litter' (= sheltering), 'resting on top of litter' or 'walking around'.

From groups of 60 isopods (15 fluorescing and 15 non-fluorescing males and 15 fluorescing and 15 non-fluorescing females each), precopula pairs were isolated once a day and checked for the visual appearance of mates to assess the mating success of fluorescing and non-fluorescing individuals.

RESULTS AND DISCUSSION

Histological studies (F. Anton-Erxleben and M. Zimmer, unpublished observations) revealed that fluorescence is the result of globular granules in Zenker cells. These cells stretch over the fifth, sixth and seventh thoracic segments and the entire abdomen (Fig. 1) in both isopods with and without conspicuous yellow stripes, and are considered homologous to tegumental glands of terrestrial isopods (Ter-Poghossian, 1909), which have been described in detail by Gorvett (1956). Zenker cells help in the storage of metabolic waste products, mainly uric acid (Zenker, 1854; Ter-Poghossian, 1909; Wägele, 1992). Despite being essentially ammonotelic, *A. aquaticus* contains large amounts of uric acid in its body, differing significantly from many other aquatic and terrestrial amphipods and isopods (Dresel and Moyle, 1950).

Assuming that fluorescing Zenker cells are biologically significant, we expected the fluorescing granules inside these cells to be excited by 'violet', 'blue' or 'green' light (400–500 nm), since the light regime under forest canopies, where *A. aquaticus* is frequently found in shaded freshwater habitats, is 'green'-dominated (Endler, 1993), and mainly light of short wavelengths ('violet', 'blue' or 'green') penetrates more deeply into the water. When screening whole-body extracts, however, essentially no fluorescence was caused by excitation at 450 nm ('blue'), 500 nm ('green') or higher wavelengths. Excitation with 'far UV' (300 nm), 'near UV' (350 nm) and 'violet' (400 nm), in contrast, caused fluorescence at 610 nm, 700 nm and ≥ 800 nm, respectively. Emitted light with wavelengths of ≥ 800 nm is not detectable by isopods, but is by many other species (e.g. fishes as potential predators). We did not detect fluorescence in homogenates of isopods without yellow stripes.

There was no obvious diurnal pattern with respect to the visibility of Zenker cells, either in response to changing temperature or to light intensity over 5 days of observation, nor were there changes during the moult cycle of individuals. In contrast, we may expect fluorescence to change in response to food, since excretory products appear to be involved. While screening for a possible nutritive origin of fluorescence, we found conspicuous yellow spots with a diameter of about 200 μm that exhibit yellowish fluorescence on maple leaf litter (*Acer pseudoplatanus*) in aquaria that contained isopods collected in the field. These globular spots with a sponge-like surface appear to be embedded in the leaf tissue (Fig. 2). When feeding on leaf litter with fluorescing spots, 40% of initially non-fluorescing isopods exhibited fluorescence after 21 days. Thus, we propose that the yellow litter spots consist of

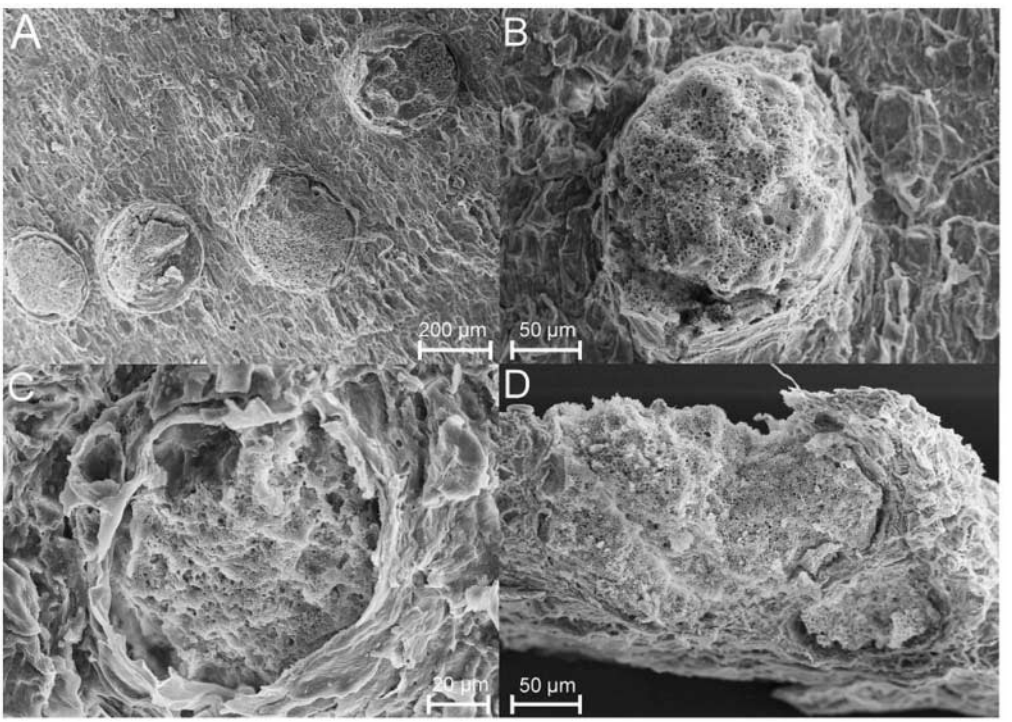

Fig. 2. Scanning electron micrograph of fluorescing spots in leaf litter. (A) Group of spots; (B) single spot in detail; (C) sponge-like spot breaking through the leaf cuticle; (D) transverse section of a litter spot, showing the sponge-like matrix that is embedded in, and breaking through, the leaf tissue and cuticle.

a fluorescing compound of unknown origin that is ingested by litter-feeding isopods and subsequently stored in Zenker cells as metabolic waste.

Fluorescing yellow stripes on the back of a brownish animal crawling on dark ground increase its visibility in shaded freshwater (authors' field observations). Three-spined sticklebacks (*Gasterosteus aculeatus*), when given a choice between one isopod with visible yellow stripes and one without visible stripes, preferentially fed on those isopods that either exhibited fluorescing Zenker cells or were manipulated with colour to carry yellowish stripes on their back, rather than on those without fluorescence or with experimentally brown-coloured backs to hide fluorescence (sign test, $n = 30$, $P < 0.01$; Fig. 3a). Similarly, when offered 10 isopods (5 with and 5 without yellow stripes) simultaneously, sticklebacks fed significantly more on those isopods that exhibited yellow stripes on their back (Fig. 3b), no matter whether isopods were painted (sign test, $n = 30$, $P < 0.05$) or not (sign test, $n = 30$, $P < 0.01$). In similar experiments with freshwater amphipods, Bakker *et al.* (1997) observed that an acanthocephalan parasite induces colour changes in its host, *Gammarus pulex*, which make it more prone to predation by sticklebacks.

Based on these and our own results, we hypothesize (hypothesis 1) changes in the isopods' visible appearance to be caused, through some unknown mechanism, by parasitic endosymbionts that need to be ingested by their final hosts together with isopods serving as prey

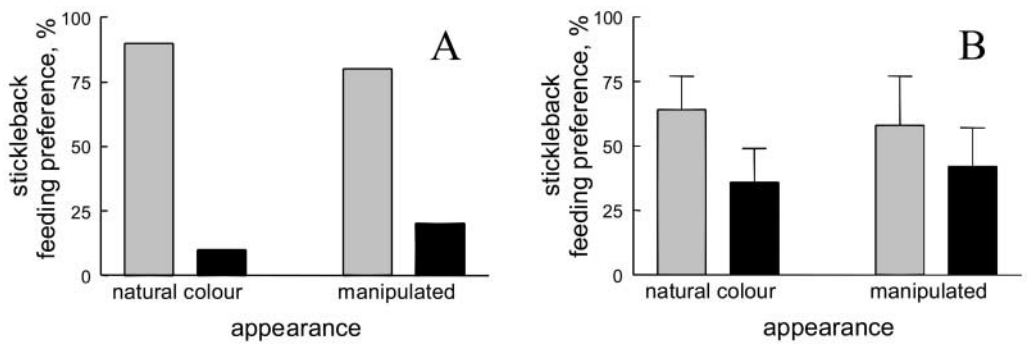

Fig. 3. Feeding preference of three-spined sticklebacks (*Gasterosteus aculeatus*) when offered isopods with (grey) and without (black) yellowish stripes on their posterior back. (A) In each test ($n = 30$), a single isopod with stripes (natural colour: Zenker cells; manipulated: visible Zenker cells covered with oil colour; cf. Bakker *et al.*, 1997) and a single one without visible stripes (natural colour: no Zenker cells visible; manipulated: oil colour; cf. Bakker *et al.*, 1997) were offered simultaneously to a single stickleback (sign test, $n = 30$, $P < 0.01$). (B) Five isopods without and five isopods with visible yellow stripes (either manipulated with oil colour or non-manipulated) were offered simultaneously; the experiment ended when five isopods had been preyed upon.

(for discussion, see Bakker *et al.*, 1997, and reference therein). Bakker *et al.* (1997) further suggested that infection by the acanthocephalan parasite causes behavioural changes in *G. pulex*, increasing the prey's susceptibility to the predator. In line with these findings, fluorescing *A. aquaticus* differed from their non-fluorescing conspecifics with respect to their behaviour, in that they spent 83 ± 9% (mean ± *s*) of their time on top of the litter layer (i.e. visible to potential predators), while isopods without fluorescence spent most of their time (96 ± 4%) hidden beneath the litter layer (Fig. 4). Whether a change in behaviour is the result of whatever causes fluorescence or, in turn, is the cause of fluorescence (for a general discussion, see Milinski, 1990; Moore and Gotelli, 1990) due to a change in feeding behaviour (e.g. on top of the litter layer *vs* beneath), remains unclear.

Prominent coloration may serve as a sexual signal that increases attractiveness to potential partners (e.g. Andersson, 1994). Thus, fluorescing Zenker cells might be involved in mate-choice and mating in *A. aquaticus*. By strongly increasing the visibility of the respective isopod and the risk of predation (Fig. 3), fluorescence might well serve as an honest signal (*sensu* Hamilton and Zuk, 1982) in courtship and mate-choice (hypothesis 2). However, even some immature isopods exhibit fluorescing Zenker cells and both females and males exhibit this character in similar percentages and strongly resemble each other (authors' observations; cf. Ter-Poghossian, 1909, for the anatomy of Zenker cells). It is, therefore, unclear if and how fluorescing Zenker cells influence mate-choice in *A. aquaticus*.

An analysis of precopula pairs in mixed populations (Fig. 5) provided evidence for assortative pairing of non-fluorescing and fluorescing isopods, respectively (χ^2 test for homogeneity, $n = 93$, $P < 0.001$). This observation, however, allows ambiguous conclusions to be drawn. The assumption of parasite-induced inferiority of fluorescing mates would suggest that most non-fluorescing males guarded non-fluorescing females in precopula, but only a few of them chose to guard fluorescing females. Most fluorescing males would then only have had access to fluorescing females, with only a few of them being able to guard

Fig. 4. Time budget of non-fluorescing (left) and fluorescing (right) *Asellus aquaticus* ($n = 15$).

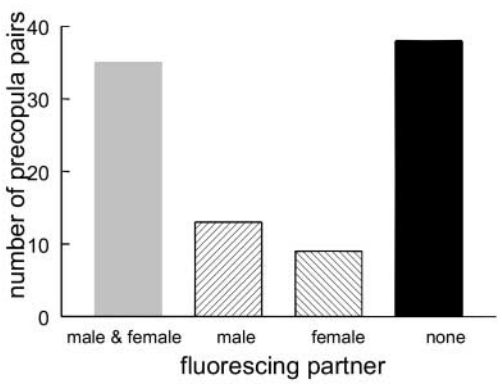

Fig. 5. Assortative pairing of fluorescing and non-fluorescing *Asellus aquaticus* in mixed populations, respectively (χ^2 test for homogeneity; $n = 93$, $P < 0.001$).

non-fluorescing females. Of course, the observed assortative pairing may also be explained by fluorescing males monopolizing fluorescing females first, so that mostly non-fluorescing females were left to mate with non-fluorescing males, if we assume fluorescence to be an honest signal (*sensu* Hamilton and Zuk, 1982) that increases predation risk and mating success.

Direct two-way choice experiments are necessary to decide beyond doubt whether fluorescence increases or decreases a mate's attractiveness. These and further long-term experiments with respect to the influence of fluorescing Zenker cells on mate-choice, as well as attempts to characterize the chemical compound that causes fluorescence and the biological origin of this compound, are in progress in our laboratory.

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