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Colour plasticity alters thermoregulatory behaviour in *Battus philenor* caterpillars by modifying the cue received

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Behaviour is an important way for animals to rapidly respond to changes in their current environment; however, over extended periods animals can also respond to environmental change via slower, developmental plasticity in other traits. This developmental plasticity could itself alter the animal's behaviour in two ways: it could change the state of the aspect of the animal's current environment that induces the behaviour (the cue), or it could change the physiology underlying production of that behaviour (the behavioural reaction norm). We tested these alternatives for two responses to temperature, colour plasticity and refuge-seeking behaviour, in pipevine swallowtail, *Battus philenor*, caterpillars. Prior research found that black caterpillars seek thermal refuges at lower ambient temperatures than red caterpillars in the field. Here, we found that the effect of colour on behaviour in the laboratory depended on how we heated the caterpillars. When warmed by radiant heat, black caterpillars sought refuge sooner than red caterpillars, as occurs in nature. In contrast, when warmed by conduction of heat, black caterpillars no longer sought refuges sooner than red caterpillars. Both colour morphs began seeking refuges at the same body temperature in both experiments, and the sensitivity of their metabolic rate to temperature was also the same. Taken together, our findings indicate that while colour does change the cue for refuge seeking, it does not change the behaviour's reaction norm. Similar cue-mediated interactions may often occur for thermoregulatory behaviour in other species.

Behaviour is a key means by which animals respond to rapid changes in their environment, particularly because most behaviours can be started or stopped almost immediately in response to environmental change (Duckworth, 2009; Snell-Rood, 2013). Behaviour is not, however, the only way for animals to respond to environmental change, particularly over extended periods. In addition to behaviour, animals often respond to environmental change with developmental plasticity in other traits, such as morphology or life history (Boersma, Spaak, & De Meester, 1998; Foster et al., 2015; Relyea, 2004). While such plastic responses are often slower, they can nevertheless still be adaptive in a stable environment where some aspect of the environment provides a reliable cue for predicting future conditions (Levins, 1968; Moran, 1992). Furthermore, slower developmental plasticity can potentially alter the expression of a more rapid behavioural response. For example, a plastic increase in gizzard size of red knots, *Calidris canutus*, is associated with a change in preferred foraging grounds towards those with harder molluscs as prey (van Gils, Dekinga, Spaans, Vahl, & Piersma, 2005).

The mechanisms by which developmental plasticity influences behaviour has, however, received little previous attention. Here, we propose two general mechanisms by which developmental plasticity can alter a behavioural response: developmental plasticity can change the cue for the behaviour, or it can change the behaviour itself (the behavioural reaction norm). First, developmental plasticity could change the information used by the animal as a cue for the state of the environment. Behaviour, like developmental plasticity, uses cues about the environment to determine an appropriate response, and if a change in another trait alters the perceived value of one of these cues, it will subsequently change the animal's behaviour (Fig. 1a). Environmental cues can be either external to the organism, such as photoperiod, or internal to the organism but still related to the environment, such as nutritional state or body temperature (Moczek, 2015; Schlichting & Smith, 2002).

Alternatively, developmental plasticity could change how behaviour responds to the environment. A diverse set of

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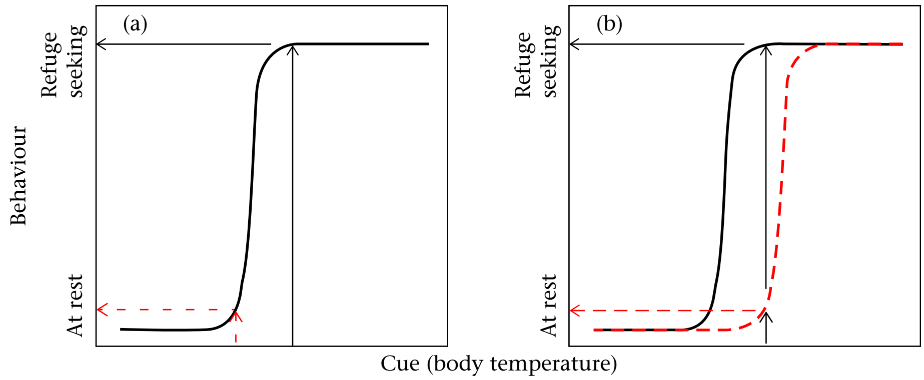

Figure 1. Hypothetical diagram of two mechanisms by which developmental plasticity could alter the expression of a behaviour, using colour plasticity and refuge-seeking behaviour in *Battus philenor* as an example. The *X* axis shows the value of the cue for the behaviour (body temperature for refuge seeking in *B. philenor*), and the *Y* axis shows the behavioural response, in this case modelled as simple on–off dichotomy with two discreet states (either at rest or refuge seeking). The curve represents the behaviour's reaction norm, whose key feature in this case is the temperature threshold at which the behaviour starts. Arrows are used to show the behavioural response associated with a particular value of the cue. (a) Hypothesis 1: developmental plasticity changes the intensity of the cue, the information used by the animal to determine whether to perform a behaviour. This is shown by two different arrows (solid and dashed), representing the different values of the cue received by animals with different developmentally plastic phenotypes, leading to different behaviours. For *B. philenor*, this would manifest as colour plasticity altering body temperature, the cue for refuge-seeking behaviour. (b) Hypothesis 2: developmental plasticity changes the reaction norm for the behaviour, changing how the animal responds to an unchanged value of the cue. This is shown by two different reaction norms (solid and dashed), representing the different response thresholds of animals raised under different conditions, leading to different responses to the same value of the cue. For *B. philenor*, this would manifest as the developmental temperature variation producing colour change also producing a linked shift in the body temperature response threshold for refuge-seeking behaviour.

physiological systems and processes produce behaviour, including neural, hormonal and muscular systems, which can themselves be developmentally plastic and change alongside other traits. The reaction norm for the behaviour—a function that relates the phenotype to the state of the environment (Scheiner, 1993)—summarizes all of these processes. Developmental plasticity in the systems underlying a behaviour will change its reaction norm, and thus the behaviour itself, independent of the immediate environment and its cues (Fig. 1b).

Temperature and thermoregulation provide an especially good ecological context for studying the relationship between behaviour and plasticity in other traits. Behaviour is arguably the most important class of traits for thermoregulation, particularly in ectotherms, due to both its speed and effectiveness (Stevenson, 1985), but plastic changes in other traits such as physiology and morphology are also often involved in thermoregulation (Angilletta, 2009; Kingsolver & Huey, 1998; Stevenson, 1985). This creates the opportunity for slower plastic changes in other traits to affect thermoregulatory behaviour via either of the two mechanisms described above. Temperature of all or part of the body is a common cue for thermoregulatory behaviour (May, 1979), in which case developmental plasticity of other traits that affect body temperature, such as colour, size and shape, should in turn alter the expression of thermoregulatory behaviour.

Alternatively, the thermoregulatory behaviour's reaction norm could be changed by developmental plastic responses to temperature. Temperature affects behaviour through both ‘kinetic’ effects—the direct physical influence of temperature on the biochemical, metabolic and physiological processes that underlie behaviour—and ‘integrated’ effects—behavioural responses to thermal information made using integrated sensory and nervous systems (Abram, Boivin, Moiroux, & Brodeur, 2017). Behavioural reaction norms depend on both of these effects, and developmental plasticity in other traits could alter the reaction norm by changing either of them. Thermal acclimation (temperature-induced change in an organism's physiology and biochemistry that alters its subsequent sensitivity to temperature; Angilletta, 2009; Huey, Berrigan, Gilchrist, & Herron, 1999) provides an example where plasticity alters the kinetic effects of temperature on behaviour. For example, changes in metabolic rate and enzyme activity following exposure to different temperatures have been associated with changes in preferred temperatures for behavioural thermoregulation in multiple ectothermic vertebrates (Berner & Puckett, 2010; Glanville & Seebacher, 2006). Alternatively, developmental plasticity in another trait could have side-effects on the animal's nervous or sensory systems, leading to a change in how they integrate sensory information on temperature, and thus their behavioural response. In both cases, the animal experiences the same body temperature (cue), but responds differently, indicating a change in the reaction norm.

To test how developmental plasticity changes thermoregulatory behaviour, we used the caterpillar of the pipevine swallowtail, *Battus philenor* L., which responds to temperature in two ways: colour change (developmental plasticity) and refuge-seeking (behaviour). *Battus philenor* caterpillars develop red body coloration when raised at warm ambient temperatures (about 36 °C or above), and black coloration at cooler temperatures (Nice & Fordyce, 2006). Placed in sunlight, red caterpillars warm more slowly and reach asymptotic body temperatures about 3 °C lower than black caterpillars (Nice & Fordyce, 2006). At particularly high temperatures, caterpillars also seek cooler locations. These ‘thermal refuges’ are typically off their low-growing host plant, on nonhost vegetation higher above the ground where air temperature is cooler (Nice & Fordyce, 2006; Nielsen & Papaj, 2015, 2017).

Nielsen and Papaj (2017) demonstrated that body coloration changes refuge-seeking behaviour: black caterpillars in field experiments moved to refuges at lower environmental temperatures than red ones and thus spent more time on refuges overall. Here, in laboratory experiments, we tested whether the effect of colour on refuge-seeking behaviour occurs because of the direct effect of body colour on body temperature (Fig. 1a) or because developmental plasticity changes the behaviour's reaction norm (Fig. 1b). Based on previous work (Nielsen & Papaj, 2015), we hypothesized that body temperature was either the cue for refuge seeking, or strongly correlated to the cue. We experimentally heated caterpillars in two ways, using either radiant heat (a strong light) or conduction (a hot plate), and assessed behaviour of the different colour morphs. Body coloration affects body temperature by changing