Animal Behaviour 108 (2015) 91–100

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Parental effects and flight behaviour in the burying beetle, *Nicrophorus vespilloides*

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ARTICLE INFO

Article history:
Received 10 March 2015
Initial acceptance 9 April 2015
Final acceptance 24 June 2015
Available online 22 August 2015
MS. number: 15-00195R

Keywords:
allometry
burying beetle
flight mill
geometric morphometrics
parental care
social evolution

Parents play a key role in determining the phenotype of their offspring. However, relatively few studies have investigated whether parents can change their offspring's behaviour in a sustained way that persists into adulthood. With experiments on the burying beetle, *Nicrophorus vespilloides*, we investigated how the developmental environment created by parents affects their offspring's wing morphology in adulthood, and the correlated effects on adult flight behaviour. Burying beetles exhibit complex biparental care, but offspring can survive without parental provisioning. By removing parents just prior to hatching, while holding the nutritional environment constant, we investigated the downstream consequences for offspring morphology and behaviour. Larvae that developed in the absence of their parents had relatively long and more slender wings than those that developed in their parents' presence. Flight mill tests revealed that flight performance was dependent on the presence of parents during development but not on wing shape. Our results demonstrate that parents have long-lasting effects on the behaviour of their offspring, by influencing the morphology and flight behaviour of their young even after they have matured into adults.

Parents play a key role in determining the phenotype of their offspring, in ways other than through standard genetic inheritance (Badyaev, 2008; Badyaev & Uller, 2009; Uller, 2012). For example, parents commonly contribute directly to the nutritional environment in which their offspring develop, both before and after birth, so influencing their offspring's body size and shape (e.g. Emlen & Nijhout, 2000). They can also influence the hormonal environment experienced during development, so further inducing developmental plasticity in the offspring's phenotype (Groothuis, Müller, von Engelhardt, Carere, & Eising, 2005). In addition, parents establish the social environment in which offspring develop, including the extent of competition with siblings (e.g. Schrader, Jarrett, & Kilner, 2015) and so potentially cause a change in the offspring's phenotype through indirect genetic effects (Moore, Brodie, & Wolf, 1997).

In these diverse ways, parents can influence attributes of their young that contribute to their offspring's fitness, such as their survival (e.g. Uller, 2012), growth rate (e.g. Rauter & Moore, 2002) and morphology (e.g. Badyaev, 2008). However, relatively few studies have investigated whether parents can also change their offspring's behavioural performance in a sustained way that persists into adulthood. Nevertheless, work on vertebrate species suggests that these effects might be widespread. For example, Groothuis et al. (2005) reported that, in birds, maternal androgens deposited inside the eggs have an effect on offspring phenotype long after fledging, resulting in individual differences in terms of territorial behaviour and sexual display. Furthermore, maternal provisioning of the egg yolk in the side-blotched lizard, *Uta stansburiana*, influences adult escape behaviour (Lancaster, McAdam, & Sinervo, 2010).

With experiments on the burying beetle, *Nicrophorus vespilloides*, we investigated how the presence of parents during posthatching development influences wing morphology when offspring reach adulthood, and the consequent effect on adult flight behaviour. *Nicrophorus vespilloides* is ideal for this work because it exhibits facultative posthatching care (Scott, 1998a; Smiseth, Darwell, & Moore, 2003), meaning that parents can be removed just before offspring hatch and the downstream consequences monitored in adulthood. In this species of burying beetle, a small vertebrate carcass is used as food resource for the developing offspring. After discovery of a suitable carcass, both parents remove fur or feathers, roll the flesh into a ball and bury the prepared carcass in an underground chamber (Pukowski, 1933; Scott, 1998b). After hatching, the offspring are fed by the parents on regurgitated

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http://dx.doi.org/10.1016/j.anbehav.2015.07.020

meat (Scott, 1998b) but are also able to self-feed on the prepared carcass (Smiseth et al., 2003). Offspring can therefore nourish themselves effectively if the parents are removed just before hatching (Schrader et al., 2015; Scott, 1998a). This quirk of natural history allowed us to manipulate the posthatching environment by removing parents and to quantify the effect on adult performance through behavioural and morphometric assays.

A key point is that changing the posthatching environment in this way does more than simply change the offspring's nutritional environment: our experiment is not simply another analysis of the effect of nutritional conditions during development on adult form and behaviour. In our experiment, larvae raised without parents lived in a near identical nutritional environment to those raised with parents, because in both treatments larvae were given a similar sized mouse carcass, prepared by parents, from which to derive nourishment. Because larvae can self-feed, even those raised without posthatching care could potentially access these resources. Thus, any differences between the two treatments are attributable not to the nutritional environment per se, but to its interaction with the social environment (i.e. the assistance parents provide to their young in obtaining resources, even when their offspring can self-feed). Furthermore, parents additionally influence the quality of the posthatching environment by changing the nature of interactions among siblings (Schrader et al., 2015), including through partial filial cannibalism (Bartlett, 1987). Our aim here was simply to determine whether (or not) parents change the posthatching developmental environment to such an extent that they impose long-term effects on the morphology and behaviour of their offspring.

We focused on flight behaviour in this study because it is essential for locating carrion, and is thus key to a burying beetle's reproductive success, yet has been little studied (but see Merrick & Smith, 2004). Vertebrate carcasses are an ephemeral and unpredictably distributed resource and they contribute to burying beetle fitness by functioning as mating arenas (Eggert, 1992) as well as a food resource for developing larvae (Pukowski, 1933; Scott, 1998b). Previous studies have shown that wing shape affects long-distance flight performance in migratory species of birds (Bowlin & Wikelski, 2008; Förschler & Bairlein, 2011), bats (Norberg & Rayner, 1987), butterflies (Breuker, Brakefield, & Gibbs, 2007; Dockx, 2007) and dragonflies (Johansson, Söderquist, & Bokma, 2009). In other insects wing shape is also directly related to individual flight ability (Betts & Wootton, 1988), reproductive success (Kölliker-Ott, Blows, & Hoffmann, 2003) and predation success (Combes, Crall, & Mukherjee, 2010). We investigated how parental care affects wing shape, and its allometric relationship with body size. We further examined how these measures are correlated with flight performance, using a flight mill for these analyses. Flight mills are widely used to investigate insect movements (e.g. Reynolds & Riley, 2002) but have more rarely been employed to address the functional morphology questions we set out here.

Although our experiment was largely exploratory, we anticipated two types of outcome. The first possibility was that removing parents after hatching would induce a poor-quality developmental environment, yielding small individuals of low quality, that were incapable of much sustained flight behaviour. An alternative possibility was that removing parents after hatching would not only reduce offspring body size at eclosion, but also induce some sort of compensatory wing development and associated flight behaviour to overcome the fitness disadvantages associated with being a small adult (cf. Moe, Brunvoll, Mork, Brobakk, & Bech, 2004; Nilsson & Svensson, 1996). When burying beetles fight for ownership of a carrion breeding resource, smaller individuals tend to lose (Hopwood, Moore, & Royle, 2014; Otronen, 1988). Perhaps small burying beetles might compensate for this disadvantage by using flight to disperse further to find carrion, taking them away from any potential rivals and so preventing the loss of a valuable breeding resource.

METHODS

Laboratory Population

Experimental individuals were from a laboratory population reared in constant conditions (21 ± 1 °C, 16:8 h light:dark cycle) in the Zoology department at Cambridge University, U.K. This population was established in 2005 and is supplemented every summer with wild-caught individuals from different sites in Cambridgeshire.

Manipulation of Posthatching Environment

In the experimental generation, we manipulated whether parents were present or absent after hatching. (These larvae were then kept until adulthood, when we analysed their flight performance.) Adult beetles were collected from the laboratory population at eclosion, housed in individual plastic boxes (12 × 8 cm and 2 cm high) filled with moist soil and fed twice a week with ca. 1 g of minced beef. Two to three weeks later, soon after the beetles reached sexual maturity, 26 pairs of unrelated males and females were placed in larger plastic boxes (17 × 12 cm and 6 cm high) half filled with moist soil, provided with a freshly thawed mouse carcass and kept in the dark to simulate natural underground conditions. Carcass mass was kept in the range of 12 ± 1 g (mean ± SE; Full Care: 12.16 ± 0.39 g; Prehatching Care: 11.91 ± 0.41 g; $W = 522.5$, $P = 0.56$; for treatments see below) to control for the variation in larval mass associated with available resources (Smiseth, Andrews, Mattey, & Mooney, 2014). Breeding boxes were kept at 22 ± 1 °C.

We assigned breeding pairs randomly to two parental care treatments: Full Care, in which parents remained in the breeding boxes with the larvae until their dispersal and Prehatching Care, in which parents were removed from the breeding boxes 53 h after pairing. Thus larvae in the Full Care treatment received both pre- and post-hatching care whereas larvae in the Prehatching Care treatment only received prehatching care, which consisted only of carcass preparation by parents. Although it is rare in nature, broods are sometimes left with no posthatching care (Müller, Braunisch, Hwang, & Eggert, 2007). Eight to nine days after pairing, we opened the breeding boxes to collect dispersing larvae and placed them in eclosion boxes where they eclosed after 16 ± 2 days. At eclosion, 40 adults (20 males and 20 females) were collected haphazardly from the 13 pairs in each care treatment, yielding 80 individuals in total for the flight assays. Although larvae were not collected at random, with so few selected from each family it is unlikely that there was any systematic bias in our sample. It is unlikely that hatching order affected our sample as it does not depend on presence or absence of parents (Smiseth, Ward, & Moore, 2007) and parents in the Prehatching Care treatment were removed after all eggs were laid but before the larvae hatched. Additionally, we sampled individuals blindly with respect to hatching order as we did not know when each larva hatched. Therefore there is no reason to expect our measurements in each treatment to be biased by hatching order.

Assay of Flight Performance

Adult beetles were kept in individual plastic boxes and maintained in our standard laboratory conditions. They were unmated, fed twice a week with ca. 1 g of minced beef and then flight-tested 20–26 days after eclosion, i.e. after reaching sexual maturity. Flight assays were performed using custom-built flight mills modified