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

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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.

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...
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 offspring). Furthermore, parents additionally influence the quality of the posthatching environment by changing the 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; Forschler & Bairlein, 2011), bats (Norberg & Rayner, 1987), butterflies (Breuer, Brakefield, & Gibbs, 2007; Dockx, 2007) and dragonflies (Johansson, Söderquist, & Bomka, 2009). In other insects wing shape is also directly related to individual flight ability (Bets & Wootton, 1988), reproductive success (Kolliker-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 flight 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, Braunsch, 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...
from a previous design (Attisano, Tregenza, Moore, & Moore, 2013) and adapted to accommodate N. vespilloides. Each flight mill was formed by a rectangular Perspex structure (40 × 40 cm and 12 cm high) with a central pivot where a magnetic bearing sustained a rotational arm. We used four circular neodymium magnets (10 mm radius × 4 mm height) to form the magnetic bearing, which provided a strong rotational support while keeping friction low. A hypodermic steel needle (370 mm length × 4.1 mm radius) was used as a rotational arm. This was bent in an L-shaped configuration with a 20 mm long curved end providing the insertion point for an entomological pin, which functioned as a tether where the focal individual was secured. Beetles were tethered using skin adhesive (Manfred Sauer UK Ltd, Northampton, U.K.) and adhesive putty (Bostik White-Tak). A square flag of foil was placed on the opposite end of the steel arm and used as a counterweight. Tethered flying beetles pushed the steel arm allowing it to rotate around its central magnetic pivot. At each revolution the foil flag interrupted the beam of an IR sensor (OPPB800, OPTEK Technology, Carrollton, TX, U.S.A.) allowing us to record the number of revolutions from which distance, speed and movement patterns were obtained.

Each behavioural assay consisted of an 8 h recording of the flight activity for each individual. All recordings were performed in environmentally controlled conditions, within an incubator at 20 °C, running on a cycle of 16:8 h light:dark synchronized with our standard rearing laboratory conditions. Beetles were tethered to the flight mills 9 h after light onset and flights were recorded until 1 h after light offset. This recording time was chosen to capture the natural variation in activity patterns showed by adult N. vespilloides at our field sites during late spring and summer months (Attisano, 2013).

The recorded flight patterns were analysed using custom-written software developed in Python (v 3.4.0): this was used to obtain the parameters of the flight performance for each beetle such as total distance travelled, longest flying bout, total flight duration, total number of flight events and average speed. We only analysed flight events in which the focal beetle flew for longer than 60 s. In total 80 individuals were flight-tested (40 Prehatching Care + 40 Full Care). Each beetle was flight-tested once.

Wing Shape Measurement Analysis

The body size of each beetle was measured using the maximum pronotum width, a standard technique for quantifying beetle body size (Beeler, Rauter, & Moore, 1999). The right wing from each individual was collected and spread between two microscope slides (Beeler, Rauter, & Moore, 1999). The right wing from each individual was collected and spread between two microscope slides and adapted to accommodate N. vespilloides. Each flight mill was formed by a rectangular Perspex structure (40 × 40 cm and 12 cm high) with a central pivot where a magnetic bearing sustained a rotational arm. We used four circular neodymium magnets (10 mm radius × 4 mm height) to form the magnetic bearing, which provided a strong rotational support while keeping friction low. A hypodermic steel needle (370 mm length × 4.1 mm radius) was used as a rotational arm. This was bent in an L-shaped configuration with a 20 mm long curved end providing the insertion point for an entomological pin, which functioned as a tether where the focal individual was secured. Beetles were tethered using skin adhesive (Manfred Sauer UK Ltd, Northampton, U.K.) and adhesive putty (Bostik White-Tak). A square flag of foil was placed on the opposite end of the steel arm and used as a counterweight. Tethered flying beetles pushed the steel arm allowing it to rotate around its central magnetic pivot. At each revolution the foil flag interrupted the beam of an IR sensor (OPPB800, OPTEK Technology, Carrollton, TX, U.S.A.) allowing us to record the number of revolutions from which distance, speed and movement patterns were obtained.

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Wing Shape Measurement Analysis

The body size of each beetle was measured using the maximum pronotum width, a standard technique for quantifying beetle body size (Beeler, Rauter, & Moore, 1999). The right wing from each individual was collected and spread between two microscope slides to visualize outline and shape of the entire wing. The wing was then digitized using a DSLR camera (Canon D1100) equipped with a wide-angle lens to visualize outline and shape of the entire wing. The wing was then digitized using a DSLR camera (Canon D1100) equipped with a wide-angle lens. The digitized images were processed using geometric morphometric techniques (Adams, Rohlf, & Slice, 2004). A total of 17 landmarks were placed on clearly identifiable morphological characters of the wing: six were used to identify the outline of the wing, three were placed on major flexion points onto which the wing is folded when encased under the elytron, five were placed at the termination of major veins of the wing and finally three were placed on clearly recognizable processes in the axillary part (Fig. 1).

Statistical Analysis

All statistical analyses were performed using R (R Core Team, 2014). Pronotum width, wing length, wing area and aspect ratio all followed the assumption of a normal distribution; thus two-way ANOVAs were used to test for differences in linear morphometric parameters between sex, care treatment and their interaction. As a preliminary screening revealed no significance of the interaction terms in any analysis, we performed a type II ANOVA, included in the package car (Fox & Weisberg, 2011), which returns the adjusted sum of squares for the main effects with the higher-level term omitted (Hector, Von Felten, & Schmid, 2010). The allometric relationships between wing dimensions and body size were calculated after log transformation of wing length, wing area and pronotum width in order to produce adimensional linear relationships. Pronotum width was squared and log transformed before plotting it against wing area to obtain comparable measurement units. The allometric relationships were analysed with major axis regression using the package lmomR2 (Legendre, 2014). The package smatr (Warton, Duursma, Falster, & Taskinen, 2012) was used to analyse the differences in slope and elevation of the major axis regressions between parental provisioning treatments and sex. The measurement units of pronotum width and aspect ratio were not comparable; thus we analysed their allometric relationship with standardized major axis regression (Warton, Wright, Falster, & Westoby, 2006) using the package smatr (Warton et al., 2012).

Total distance travelled, total flight duration, average speed and duration of the longest flying bout did not follow the assumption of a normal distribution; thus we used a Wilcoxon rank sum test to assess how these measures were affected by the two parental care treatments. However, given the high correlation of some of these parameters, we assessed general flight performance with a principal component analysis (PCA) using the package psych (Revelle, 2014). The PCA included all the values obtained from the analysis of the recorded flight tracks and it returned two main components that accounted for 80% of the variance (PC1 = 58%, eigenvalue = 2.89; PC2 = 23%, eigenvalue = 1.13).

The first component best explained the variance associated with total distance, duration of the longest flying bout and total flight duration; it was thus named ‘performance’ component because it explained the general flight performance and the propensity of each individual to engage in a sustained flight event for a prolonged period of time. The second component was almost entirely associated with total number of flight events and with duration; it was...
thus named ‘restlessness’ because it accounted for repeated movement patterns within individuals. Average speed showed very similar loadings on both components and was thus not included in the PCA interpretation. We used the ‘performance’ and ‘restlessness’ components as dependent variables in a linear model that included aspect ratio, pronotum width, care treatment and the interactions aspect ratio × care and pronotum width × care as main effects. Sex was included in the model as covariate.

Variation in wing shape was analysed using the relative position of the landmark coordinates, after keeping mathematically constant the effect of nonshape variation deriving from position, orientation and scale (Bookstein, 1991). Landmark coordinates were digitized on the wing photos using the multipoints tool in Fiji (Schindelin et al., 2012). The data set containing the landmark coordinates was then analysed with a full Procrustes fit using MorphoJ (Klingenberg, 2011). The Procrustes approach extracts shape information from coordinate data eliminating reflection, variation in scale, position and orientation. Wing specimens were translated to the origin, scaled to a unit centroid size and rotated around their shared centroid to minimize the sum-of-squared deviations of the coordinates from all specimens to achieve an optimal fit to the consensus (average) configuration. A PCA was used to visualize the main variation in wing shape features. Finally, we performed a cross-validated discriminant analysis with 10,000 permutation tests (to overcome the influence of a small sample size) using treatment and sex as main effects.

Ethical Note

Individuals used as parents in both parental care regimes were returned to our standard laboratory housing conditions after experimental manipulation or larvae collection. Larvae in their dispersal stage were gently collected and chilled for 20 s in a petri dish placed in direct contact with ice. This facilitated tethering operations where a drop of nontoxic skin glue was applied to the pronotum. After the flight test adults were placed in small individual plastic containers with moist soil and adequate food. No beetles showed any signs of stress after the flight test and all continued their natural behaviour. No beetle was harmed or accidentally killed during the tethering procedure. One day after the flight test individuals were killed by placing them in a –20 °C freezer overnight.

RESULTS

Wing Shape and Size

Sex

The sexes did not differ significantly in pronotum width (mean ± SE; males: 4.61 ± 0.07 mm; females: 4.52 ± 0.07 mm; \( F_{78} = 0.829, P = 0.365 \), aspect ratio (mean ± SE; males: 3.17 ± 0.02; females: 3.15 ± 0.02; \( F_{78} = 0.429, P = 0.515 \), wing length (mean ± SE; males: 14.31 ± 0.2 mm; females: 14.06 ± 0.2 mm; \( F_{78} = 0.759, P = 0.386 \)) or wing area (mean ± SE; males: 63.33 ± 1.87 mm²; females: 62.37 ± 1.87 mm²; \( F_{78} = 0.535, P = 0.467 \)).

Posthatching environment

The posthatching environment had a significant effect on wing shape, with the wings of Prehatching Care beetles having a greater aspect ratio than those of Full Care beetles (mean ± SE; Prehatching Care: 3.19 ± 0.02; Full Care: 3.13 ± 0.02; \( F_{1,78} = 5.601, P = 0.02 \)). However, the posthatching environment had no significant effect on pronotum width (mean ± SE; Prehatching Care: 4.58 ± 0.06 mm; Full Care: 4.55 ± 0.09 mm; \( F_{1,78} = 0.129, P = 0.72 \), wing length (mean ± SE; Prehatching Care: 14.46 ± 0.15 mm; Full Care: 13.93 ± 0.23 mm; \( F_{1,78} = 3.695, P = 0.058 \)) or wing area (mean ± SE; Prehatching Care: 66.00 ± 1.49 mm²; Full Care: 62.84 ± 2.19 mm²; \( F_{1,78} = 1.413, P = 0.238 \)) nor did we find a significant interaction between posthatching environment and sex in any of our analyses.

Allometric scaling

Wing dimensions were disproportionately large in smaller individuals. In other words, wing length (major axis regression = 1.331 ± 0.871x, \( P = 0.001 \) and wing area (major axis regression = 1.29 ± 0.944x, \( P = 0.001 \)) showed a hypoallometric relationship with body size. The slope of the allometric relationship was significantly smaller than 1 for wing length (ratio test\( t_{78} = -0.347, P = 0.002 \)) but not for wing area (ratio test\( t_{78} = -0.154, P = 0.162 \)).

Although the slopes of these allometric relationships did not differ significantly between the sexes (likelihood ratio test: wing length: \( \text{LRT}_1 = 0.878, P = 0.349 \); wing area: \( \text{LRT}_1 = 0.806, P = 0.369; \) Fig. 2a, b), the intercept was greater for females for both wing length (Wald test: \( W_1 = 30.74, P < 0.0001; \) Fig. 2a) and wing area (\( W_1 = 25.33, P < 0.0001; \) Fig. 2b).

Similarly, the gradient of the allometric relationship between wing length and body size did not differ significantly between the two parental care treatments (wing length: \( \text{LRT}_1 = 0.703, P = 0.402; \) Fig. 2c), nor did the gradient of the allometric relationship between wing area and body size (wing area: \( \text{LRT}_1 = 0.235, P = 0.628; \) Fig. 2d). However, the intercept was greater for Prehatching Care individuals in each case (wing length: \( W_1 = 21.84, P < 0.0001; \) wing area: \( W_1 = 7.741, P = 0.005; \) Fig. 2c, d).

These hypoallometric relationships resulted in wing shape changes with body size, with larger individuals possessing wings that were shorter and broader and smaller individuals bearing wings that were longer and more slender (Fig. 3). Specifically, there was a negative correlation between aspect ratio and pronotum width (standardized major axis regression = 3.979–0.186x, \( P < 0.001, R^2 = 0.363 \)). The intercept of the linear relationship did not differ significantly between the sexes (Wald test: \( W_1 = 0.104, P = 0.746 \), while Prehatching Care beetles had a higher intercept than Full Care beetles (\( W_1 = 6.53, P = 0.011 \)). Similarly, the gradient of the linear relationship did not differ between the sexes (likelihood ratio test: \( \text{LRT}_1 = 0.053, P = 0.818 \)) but was greater for Prehatching Care individuals than for Full Care individuals (\( \text{LRT}_1 = 5.667, P = 0.017 \)). This suggests that the presence of parents after hatching contributed more than an individual’s sex to final wing shape.

Geometric morphometric analyses

The geometric morphometric analysis confirmed that the quality of parental care significantly influenced wing shape. The first three PCA components explained 55% of the total variance in wing shape (PC1 = 28.82%, PC2 = 15.93%, PC3 = 11.20%) and the plot of the first two components was able to graphically discriminate between the two treatment groups (Fig. 4). Although there was a significant effect of posthatching environment on wing shape (discriminant analysis: Procrustes distance = 0.007, \( P = 0.015 \)) it was impossible to discriminate shape variation between males and females (discriminant analysis: Procrustes distance = 0.005, \( P = 0.389 \)). Prehatching Care beetles had longer and more slender wings than Full Care beetles (Fig. 5).
Flight Performance

Nicrophorus vespilloides individuals engaged in a wide range of flight schedules, with some showing only very short bursts of flying activity while others engaged in longer and more sustained flights. The general outcome was a wide distribution of distances (68 m–26 km) and flight duration (61 s–6.5 h).

Effect of parental care
Prehatching Care beetles covered less distance (mean ± SE; males: 4500.00 ± 726.71 m; females: 10 895.38 ± 1143.62 m; W = 1201.5, P < 0.001; Fig. 6a), spent less time in flight (mean ± SE; males: 7353.35 ± 941.94 s; females: 14 440.30 ± 1340.53 s; W = 1188, P < 0.001; Fig. 6b), flew more slowly (mean ± SE; males: 0.61 ± 0.03 m/s; females: 0.81 ± 0.03 m/s; W = 1195.5, P < 0.001; Fig. 6c), and initiated fewer flight events during the 8 h recording period (mean ± SE; males: 5.65 ± 0.93; females: 13.32 ± 1.83; W = 1222, P < 0.0001; Fig. 6d). However, there was no effect of the posthatching environment on the duration of the longest flying bout (mean ± SE; males: 4799.95 ± 776.07 s; females: 7278.85 ± 973.4 s; W = 971, P = 0.101).

Sex
Males tended to fly longer distances than females (mean ± SE; males: 9562.16 ± 1245.79 m; females: 6093.37 ± 872.73 m; W = 586.5, P < 0.044). However, both sexes spent a similar amount of time in flight (mean ± SE; males: 12 541.62 ± 1366.59 s; females: 9481.53 ± 1180.81 s; W = 625, P = 0.101), initiated a similar number of flights (mean ± SE; males: 10.84 ± 1.83; females: 8.32 ± 1.33; W = 635.5, P = 0.125) and travelled at similar average speeds (mean ± SE; males: 0.74 ± 0.04 m/s; females: 0.68 ± 0.04 m/s; W = 664, P = 0.206). The sexes also did not differ in the duration of their longest flying bouts (mean ± SE; males: 6455.65 ± 969.77 s; females: 5681.23 ± 840.56 s; W = 733.5, P = 0.553).

Wing morphology and body size
The posthatching environment alone affected the ‘performance’ component while none of the other factors or interactions significantly explained the variation in the overall flight performance and ‘restlessness’ component (Table 1). Full Care individuals had overall higher performance scores than Prehatching Care individuals (Fig. 7). Both sexes showed similar measures of ‘performance’ and ‘restlessness’.

Figure 2. The relationship between wing dimensions and body size, by sex and parental care treatment. Major axis regressions lines are shown and each data point represents a different beetle. (a) The relationship between wing length and pronotum width for males and females. (b) The relationship between wing area and pronotum width for males and females. (c) The relationship between wing length and pronotum width for Full Care and Prehatching Care parental regimes. (d) The relationship between wing area and pronotum width for Full Care and Prehatching Care parental regimes.
DISCUSSION

Our results show that alternative larval environments, shaped socially by the presence or absence of parents, generate morphological variation in adult N. vespilloides wing shape. Smaller individuals (Fig. 3), and those that received no posthatching care (Fig. 5), developed wings with a greater aspect ratio and thus had relatively longer and more slender wings than larger individuals, and those that received posthatching care. Furthermore, the presence of posthatching care explained overall flight performance in adulthood (Fig. 7). Our study therefore shows that the social developmental environment has long-lasting downstream effects on both adult morphology and behaviour.

Parental Environment, Wing Morphology and Flight Behaviour

Wing shape is known to vary with different environmental parameters such as latitude (Azevedo, James, McCabe, & Partridge, 1998; Gilchrist, Azevedo, Partridge, & O’higgins, 2000), temperature (Debat, Bégin, Legout, & David, 2003) and habitat structure (Outomuro, Dijkstra, & Johansson, 2013; Vanhooydonck, Herrel, Gabela, & Podos, 2009) but few other studies have considered how it might be affected by the developmental environment shaped by parents. Given the likely challenging conditions that result from the absence of posthatching parental care, it is possible that the morphological variation that we report here might be due to mechanisms that are activated during developmental stress. For example, wing shape morphology in Drosophila melanogaster is affected by temperature (Debat et al., 2003) and crowding (Bitner-Mathé & Klaczko, 1999) during development. Mechanisms such as the expression of Hsp proteins and a change in the cell size/number trade-off are involved in the stress response that changes wing shape (De Moed, De Jong, & Scharloo, 1997). Food deprivation may further influence wing size by contributing to variation in cell size or number (James, Azevedo, & Partridge, 1997). The difference in wing shape between our two parental care treatments may involve similar stress response mechanisms, although this remains a subject for future work.

The removal of parents just prior to hatching during development also had downstream consequences for aspects of the burying beetle’s flight behaviour (Figs. 6 and 7, Table 1). Individuals that experienced no provisioning after hatching developed into adults that flew less far, spent less time in flight, flew at slower speeds and initiated fewer flights than those that were provisioned after hatching. Work on other insects suggests that these differences could be attributable to food deprivation (Pellegroms, Van Dongen, Van Dyck, & Lens, 2009). This might also be true for N. vespilloides, but seems unlikely to be the full story because the presence of parents after hatching did not increase adult body size in our experiment. This is a surprising result, at first sight, seemingly at odds with the results obtained from similar manipulations on N. vespilloides in previous work (e.g. Eggert et al., 1998; Smiseth et al., 2007) and so worth some discussion. A key point is that there are important differences in the methods between our study and those conducted previously. Whereas the earlier studies (Eggert et al., 1998; Smiseth et al., 2007) manipulated brood size relative to carcass size, so changing the density of larvae on the carcass, we did not. We have recently shown that at low densities, larvae perform much worse when parents are removed than when they are present (Schrader et al., 2015). However, at higher densities, like those in the experiment we report here, larvae perform equally well whether their parents are present or absent (Schrader et al., 2015). This potentially explains the discrepancy between the earlier studies (Eggert et al., 1998; Smiseth et al., 2007) and our results.

Sex, Wing Morphology and Flight Behaviour

We found that females had a greater wing length and wing area for their body size. Yet, despite differences in wing allometries, we found no corresponding sex differences in wing shape and flight behaviour, excluding a marginal difference in total flight distance
**Figure 5.** The variation in wing shape between beetles developed as larvae in Full Care or Prehatching Care conditions after a discriminant analysis with parental care treatment as the main effect. The mean shape for each group is visualized through a wireframe of straight lines connecting the landmarks used for the geometric morphometric analysis. Numbers indicate the landmarks used to perform the geometric morphometric analysis.

**Figure 6.** Frequency distributions of (a) total flight distance, (b) total flight duration, (c) average flight speed and (d) total number of flight events. Black bars represent individuals derived from the Full Care parental regime, white bars represent individuals derived from the Prehatching Care parental regime and light grey bars represent the overlapping distribution of the two care regimes.
between males and females. In other insects, it is not uncommon for females to have a larger wing area because they have a larger body mass than males (Breuker et al., 2007). This translates into a higher wing loading (body mass/wing area), which can affect flight behaviour (Betts & Wootton, 1988). We did not measure body mass in this study and so we do not know whether wing loading differed between males and females. Even if we had quantified wing loading, it is unlikely to affect flight performances in a flight mill because tethered experimental subjects do not have to bear much of their body mass during flight (Auerswald, Schneider, & Gade, 1998). This might explain why we could not detect any sex differences in flight behaviour and it is possible that studies employing tests in free-flight conditions could reveal hitherto uncovered sex differences in flight performance. Nevertheless, our flight data match analyses of the dispersal behaviour shown by American burying beetles, Nicrophorus americanus (Creighton & Schnell, 1998). Using a mark recapture method the authors estimated movement patterns and found that although individuals showed a wide variety of displacement patterns and movement ability, this variation could not be explained by sex (Creighton & Schnell, 1998).

Wing Morphology, Posthatching Care and Flight Performance

We found that smaller beetles had longer and more slender wings (i.e. a higher aspect ratio) than beetles that were tended by their parents. In larger animals, and those that depend on gliding flight, a high aspect ratio is generally correlated with a high degree of movement (Bowlin & Wikelski, 2008; Dockx, 2007; Johansson, Söderquist, & Bokma, 2009). However, hovering flyers such as burying beetles face very different aerodynamic forces (Ennos, 1989). During hovering flight, high aspect ratio wings may offer greater resistance than low aspect ratio wings (Ellington, 1984). Furthermore, individuals whose wings have a high aspect ratio also show a decrease in wing beat frequency (Berwaerts et al., 2006; Husak, Ribak, Wilkinson, & Swallow, 2011). However, we found that wing aspect ratio did not influence the overall flight performance in a flight mill.

Flight performance was contingent on whether parents were present after hatching: individuals’ flight performance was poorer if they developed without parents (Fig. 7). One possible explanation is that flight performance measured in a mill is a function of the amount of energy reserves and the rate of energy consumption. Perhaps energy reserves were greater in those individuals that received posthatching parental care.

Body size did not affect the overall flight performance and both large and small individuals were equally able to engage in similar patterns of repeated flight activity. The similarity in flight performance may be an artefact of tethering individuals in a flight mill. Without the constraint of having to lift a greater body mass a possible size effect on flight performance may have been concealed. However, previous work with flight mills on other insect species showed that a larger body size may offer a larger store of resources to fuel repeated bouts of flight (Bruzzone, Villacide, Bernstein, & Corley, 2009) or that larger individuals fly for longer (Attisano et al., 2013). Free-flight tests in variable wind and temperature conditions are now needed to determine whether body size affects flight performance in N. vespilloides.

Is Developmentally Induced Variation in Flight Behaviour Adaptive?

Finally, our data provide mixed support for the hypothesis that resource allocation to wing development, and flight performance, varies adaptively according to the developmental environment. On the one hand, we found that smaller individuals (and those that experienced no posthatching care) had disproportionately larger wings for their body size, which were also longer and more slender. Since wing morphologies do not scale perfectly with body size, this suggests that resources are allocated differently to different body parts according to overall levels of larval nutrition. However, our behavioural assays do not support the hypothesis that this resource allocation is adaptive, or at least not in the way that we imagined (and assuming that measurements using our flight mills correspond with performance in free flight). Rather than dispersing further to avoid larger rivals, our analyses instead suggest that individuals that received no posthatching care (i.e. those with a high wing aspect ratio) are less capable of sustained flight and are also

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**Table 1**

|                  | Performance         | Restlessness       |
|------------------|---------------------|--------------------|
|                  | Estimate | SS      | $F_{1, 73}$ | $P$     | Estimate | SS      | $F_{1, 73}$ | $P$     |
| AR               | -0.213   | 0.439   | 0.601      | 0.441   | -2.831   | 0.965   | 0.19      | 0.664   |
| Care             | -5.947   | 18.350  | 25.126     | <0.001  | -11.379  | 0.196   | 0.19      | 0.664   |
| PW               | 0.495    | 2.402   | 3.289      | 0.074   | -0.224   | 0.02    | 0.019     | 0.891   |
| Sex              | 0.337    | 2.203   | 3.016      | 0.087   | -0.003   | 0.000   | 0.002     | 0.989   |
| AR* care         | 1.758    | 0.499   | 0.684      | 0.411   | 2.603    | 1.095   | 1.063     | 0.306   |
| PW* care         | -0.132   | 0.051   | 0.069      | 0.792   | 0.672    | 1.314   | 1.276     | 0.262   |

Performance (PC1) explains the variation associated with total distance, total duration and duration of the longest flying bout. Restlessness (PC2) explains the variation associated with the number of flight events. PW = pronotum width, AR = wing aspect ratio.
less likely to engage in flights. By not dispersing as far as their potential rivals, it may be that these individuals can still avoid competition for a carcass with beetles that had a more favourable developmental environment. Or it may simply be that these individuals are constrained by a lack of resources during development to move far. With regard to body size, rather than compensating through dispersal behaviour for their small size, and inferior competitive ability, perhaps small individuals may adaptively allocate resources among body structures to promote success in their alternative mating strategies (Eggert, 1992). Perhaps in burying beetles, the reallocation of resources to egg and sperm production is a more effective way to promote fitness than investment in structures to promote dispersal. This possibility remains to be investigated.

In conclusion, we have shown that the social environment in which offspring develop can have long-lasting effects on their morphology and flight behaviour as adults, even when the nutritional resources potentially available to offspring are held constant experimentally. It would be interesting to investigate in more detail whether these downstream changes arise because parents enable offspring to utilize resources in their nutritional environment more effectively, or whether parents modify the extent of competition among their young for the limited resources on the carcass.

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