



Research

Cite this article: Pilakouta N, Smiseth PT. 2016 Maternal effects alter the severity of inbreeding depression in the offspring. *Proc. R. Soc. B* **283**: 20161023. <http://dx.doi.org/10.1098/rspb.2016.1023>

Received: 10 May 2016

Accepted: 23 August 2016

Subject Areas:

behaviour, ecology, evolution

Keywords:

body size, burying beetle, maternal effects, *Nicrophorus vespilloides*, offspring fitness, parental care

Author for correspondence:

Natalie Pilakouta

e-mail: n.pilakouta@gmail.com

Electronic supplementary material is available online at [doi:10.6084/m9.figshare.c.3461760](https://doi.org/10.6084/m9.figshare.c.3461760).

Maternal effects alter the severity of inbreeding depression in the offspring

Natalie Pilakouta and Per T. Smiseth

Institute of Evolutionary Biology, University of Edinburgh, Edinburgh, UK

NP, 0000-0001-8503-520X

A maternal effect is a causal influence of the maternal phenotype on the offspring phenotype over and above any direct effects of genes. There is abundant evidence that maternal effects can have a major impact on offspring fitness. Yet, no previous study has investigated the potential role of maternal effects in influencing the severity of inbreeding depression in the offspring. Inbreeding depression is a reduction in the fitness of inbred offspring relative to outbred offspring. Here, we tested whether maternal effects due to body size alter the magnitude of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. We found that inbreeding depression in larval survival was more severe for offspring of large females than offspring of small females. This might be due to differences in how small and large females invest in an inbred brood because of their different prospects for future breeding opportunities. To our knowledge, this is the first evidence for a causal effect of the maternal phenotype on the severity of inbreeding depression in the offspring. In natural populations that are subject to inbreeding, maternal effects may drive variation in inbreeding depression and therefore contribute to variation in the strength and direction of selection for inbreeding avoidance.

1. Introduction

Inbreeding results from matings between relatives and can lead to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed [1]. As a result, inbreeding is commonly associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression. Inbreeding depression is an important issue in evolutionary biology and ecology because it can exert strong selection pressures on dispersal strategies, mating systems, reproductive strategies, and social behaviours [2]. Furthermore, inbreeding depression is a growing conservation concern, given that increasing rates of habitat loss and habitat fragmentation can increase the likelihood of inbreeding [3,4], thereby contributing to higher local extinction rates [5]. Even though there is abundant evidence for inbreeding depression across a wide range of animal and plant taxa, the severity of inbreeding depression can vary dramatically both among and within species [5,6]. Understanding the factors that underlie this variation could have implications for the conservation of many endangered populations, yet these dynamics are still not well understood. Earlier work has proposed that this variation may be partly attributed to differences in the physical or social environment [7–9]. Environmental stresses, such as starvation and competition, tend to exacerbate inbreeding depression [8,10], whereas benign conditions may moderate inbreeding depression [11,12].

We have recently shown that inbreeding depression is more severe in the absence of maternal care, suggesting that the presence of the mother during offspring development can buffer against inbreeding depression [12]. However, it is still unknown whether such a buffering effect depends on the mother's phenotype. Maternal condition may affect the quantity or quality of care provided to the offspring [13–17], so we might expect the severity of inbreeding depression to be influenced by maternal traits such as body size, age,

nutritional condition, and health status. This type of a causal influence of the maternal phenotype on the offspring phenotype would represent a maternal effect [18]. Even though the mechanisms and consequences of maternal effects have been studied extensively [18,19], previous work has overlooked the potential role of maternal effects in the context of inbreeding depression.

Here, we use the burying beetle *Nicrophorus vespilloides* to examine whether maternal body size, an important component of the maternal phenotype, affects the severity of inbreeding depression in the offspring. Burying beetles are a highly suitable study system for addressing this question. They have facultative biparental care, and male removal has no effect on offspring fitness under laboratory conditions [20], allowing us to focus on maternal effects. Moreover, we have previously shown that there is severe inbreeding depression in this species, with respect to survival at the larval and pupal stages, as well as adult lifespan [12,21,22]. We have also shown that inbreeding depression in the offspring is less severe when the mother is present than when she is removed [12]. Given that small females provide less post-hatching care than large females [16,17], we hypothesized that inbred offspring would suffer a greater reduction in fitness (compared with outbred offspring) if they had a small mother than if they had a large mother. To test this hypothesis, we used a 2×2 factorial design with the following treatment groups: (i) a large female with outbred offspring, (ii) a small female with outbred offspring, (iii) a large female with inbred offspring, and (iv) a small female with inbred offspring. Because inbreeding depression affects traits across the entire life cycle in this species [12,21,22], we measured several offspring fitness traits at different life stages: (i) hatching success, (ii) larval mass at dispersal, (iii) survival rate from hatching to dispersal, (iv) survival rate from dispersal to eclosion, and (v) lifespan after eclosion.

2. Material and methods

(a) Study species

Burying beetles breed on carcasses of small vertebrates. Parents bury the carcass and lay the eggs in the soil around it [23]. They prepare the carcass by removing any fur or feathers and apply antimicrobial secretions to suppress bacterial and fungal growth [23–25]. After hatching, larvae crawl to the carcass and start feeding in a crater created by the parents. The larvae can self-feed, but parents also provision larvae with predigested carrion. In addition, parents defend the brood from predators and conspecific competitors [26]. Although both parents typically provide care, females stay on the carcass for longer and spend more time provisioning food to the larvae [17,20,27–29]. The larvae disperse from the carcass about 5 days after hatching, which corresponds to the end of the parental care period. They pupate about 10 days after dispersal and eclose as adults about 10 days after pupation.

(b) Beetle husbandry

We used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of second-generation beetles from lines originally collected in Edinburgh, UK. They were housed individually in transparent plastic containers ($12 \times 8 \times 2$ cm) filled with moist soil and kept at 20°C and constant light. All

non-breeding adults were fed small pieces of raw organic beef twice a week.

(c) Experimental design

In the first part of our experiment, we generated small and large beetles. Because adult body size is determined by larval mass at the dispersal stage [30,31], it is possible to generate different-sized beetles by removing larvae from the carcass at different times after hatching [16,17,32,33]. Thus, for each of 89 broods, we removed third-instar larvae weighing 100–150 mg and 200–250 mg to generate small and large adults, respectively. The main advantage of this method was that it allowed us to generate small and large females that were full siblings. We were thus able to remove any potential confounding genetic effects that might have arisen if we had selected small and large individuals from our stock population. After each small or large larva was removed from the carcass, it was placed in an individual container ($12 \times 8 \times 2$ cm) filled with moist soil. At eclosion, we measured the pronotum width of all female beetles. As intended, there was a substantial difference in the mean (\pm s.d.) pronotum width of females from the two groups: 4.04 (\pm 0.24) mm for small females and 5.33 (\pm 0.24) mm for large females. There was also no overlap in the range of pronotum widths for small (3.50–4.59 mm) and large females (4.99–6.00 mm). Steiger [16] used similar size classes: 3.97 (\pm 0.21) mm for small females and 5.54 (\pm 0.23) mm for large females. These categories were based on the size range observed in both the laboratory population and beetles collected in the field [16].

In the second part of our experiment, we used a 2×2 factorial design with the following treatment groups: (i) a large female with outbred offspring, (ii) a small female with outbred offspring, (iii) a large female with inbred offspring, and (iv) a small female with inbred offspring. To produce outbred offspring for treatments (i) and (ii), we paired outbred virgin beetles that had no common ancestors for at least two generations. To produce inbred offspring for treatments (iii) and (iv), we paired outbred virgin beetles that were full siblings. All male and female parents were mated within 15 days after reaching sexual maturity (i.e. 10–25 days after eclosion). On the day of mating, we measured each female's prebreeding mass, which was later used to estimate the female's mass change over the breeding attempt (see below). Each experimental pair ($n = 276$) was placed in a transparent plastic container ($17 \times 12 \times 6$ cm) filled with 1 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (24–26 g). After mating, we checked the containers twice a day for the presence of eggs. As soon as the first eggs were laid, we removed the male from the container. In this species, the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under laboratory conditions [20]. Right before larvae started hatching, we recorded the number of eggs laid (clutch size) by counting the number of eggs visible at the bottom of the transparent breeding box [33,34]. Because each box contained a very thin layer of soil, the number of eggs at the bottom of the box was very close to the actual clutch size [34].

When all larvae had dispersed from the carcass, we weighed the female again. By subtracting each female's prebreeding mass from her postbreeding mass, we calculated her mass change over the breeding attempt, as a measure of somatic investment and thus allocation to future reproduction [32,35]. Females were then transferred to individual containers ($12 \times 8 \times 2$ cm) filled with moist soil. They were checked twice a week until death to determine their postbreeding lifespan, as a measure of their residual reproductive value [33].

At the dispersal stage, we also recorded the number of unhatched eggs visible at the bottom of the box, the number of

Table 1. Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small), and their interaction on female reproductive decisions (clutch size and mass change) and residual reproductive value (postbreeding lifespan). We provide information on the test statistic (F or LR χ^2 for linear models or generalized linear models, respectively) and p -value for each variable. Statistically significant p -values are indicated in italics.

	offspring inbreeding status		female size		interaction	
	F /LR χ^2	p -values	F /LR χ^2	p -values	F /LR χ^2	p -values
clutch size	1.90	0.17	263	<0.0001	0.03	0.86
female mass change (%)	0.11	0.74	11.1	<0.01	0.20	0.65
female postbreeding lifespan (days)	0.09	0.77	9.7	<0.01	2.21	0.14

surviving larvae, and the total mass of the brood. By subtracting the number of unhatched eggs from the clutch size recorded earlier, we estimated the number of eggs that hatched. We then divided the number of eggs that hatched successfully by clutch size to calculate hatching success. We also calculated the average larval mass in each brood by dividing total brood mass by the number of larvae. We placed all larvae from each brood into large transparent containers ($17 \times 12 \times 6$ cm) filled with moist soil. Approximately 20 days later, we recorded the number of individuals that eclosed successfully from each brood to calculate the survival rate from dispersal to eclosion. At this stage, up to six beetles from each brood were placed into individual containers ($12 \times 8 \times 2$ cm). We tracked the adult lifespan of these offspring ($n = 872$) by checking them twice a week until death. The sample sizes for matings with at least one offspring surviving until eclosion were as follows: $n = 46$ for large females with outbred broods, $n = 54$ for small females with outbred broods, $n = 40$ for large females with inbred broods, and $n = 43$ for small females with inbred broods.

(d) Data analysis

All analyses were performed using R v. 3.2.3. We used linear models for continuous traits with normally distributed random errors (average larval mass, adult offspring lifespan, female mass change, and female postbreeding lifespan). For discrete traits, we used generalized linear models fitted with a Poisson error distribution (clutch size). For proportion data, we used generalized linear models fitted with a binomial distribution (survival to dispersal and survival to eclosion) or a binomial distribution corrected for overdispersion (hatching success). Proportion data were entered into the models using the 'cbind' function. In all of these models, we analysed absolute differences rather than log-transformed measures [36], as relative measures of inbreeding depression are potentially biased [37].

All models included the following factors: offspring inbreeding status (outbred or inbred), maternal body size (large or small), and the interaction between the two. A statistically significant interaction would suggest that maternal body size affects the severity of inbreeding depression in the offspring (i.e. the extent to which inbred offspring perform less well compared with outbred offspring). Carcass size was added as a covariate in all models, because the amount of resources available may influence female reproductive decisions and offspring performance. Indeed, we found that females laid more eggs on larger carcasses (LR $\chi^2 = 8.87$, $p < 0.01$), and larvae had a higher survival rate on larger carcasses (LR $\chi^2 = 6.47$, $p = 0.01$). There was also a non-significant trend for females to gain more mass on larger carcasses ($F = 3.20$, $p = 0.08$). In addition, we added maternal age as a covariate in all models, because it can influence female reproductive decisions and offspring performance. Accordingly, we found that younger females laid fewer eggs (LR $\chi^2 = 8.56$, $p < 0.01$) and their offspring had a higher survival

rate from hatching to dispersal (LR $\chi^2 = 28.8$, $p < 0.0001$). Lastly, we added sex as a covariate in the model for adult lifespan of the offspring and found that male offspring had a shorter lifespan after eclosion than female offspring ($F = 9.16$, $p < 0.001$). Decisions on which covariates to include in the final models were based on Akaike information criterion (AIC) scores.

3. Results

(a) Effects of inbreeding

There was no difference in clutch size, mass change, or postbreeding lifespan between females that were mated to their brothers and females that were mated to unrelated males (table 1; electronic supplementary material, table S1). However, inbreeding had significant effects on the offspring's fitness (table 2; electronic supplementary material, table S1). Inbred larvae suffered substantial inbreeding depression in three of the five traits we measured: survival from hatching to dispersal, survival from dispersal to eclosion, and adult lifespan (figure 1). There was no evidence for inbreeding depression in hatching success or larval mass at the dispersal stage (table 2; electronic supplementary material, table S1).

(b) Effects of female body size

Small females laid fewer eggs, gained relatively less mass over the breeding attempt, and had a shorter postbreeding lifespan than large females (table 1; electronic supplementary material, table S1). Small females also produced larvae that had a lower mass at the dispersal stage than larvae of large females (table 2; electronic supplementary material, table S1). However, female body size had no effect on hatching success, survival to dispersal, survival to eclosion, or adult lifespan of the offspring (table 2; electronic supplementary material, table S1).

(c) Interaction between inbreeding and female size

There was a significant interaction between offspring inbreeding status and female size on survival to dispersal (table 2). This interaction reflected that offspring of large females suffered a greater reduction in fitness due to inbreeding than offspring of small females (figure 2). In other words, inbreeding depression in survival to dispersal was more severe for offspring of large mothers than those of small mothers (figure 1). There was no such interaction on hatching success, larval mass, survival to eclosion, or offspring lifespan after eclosion (table 2). Similarly, there was no interaction on female reproductive decisions or residual reproductive value (table 1).

Table 2. Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small), and their interaction on offspring fitness traits. Survival to dispersal refers to the offspring survival rate from hatching to dispersal, and survival to eclosion refers to the offspring survival rate from dispersal to eclosion. We provide information on the test statistic (F or LR χ^2 for linear models or generalized linear models, respectively) and p -values for each variable. Statistically significant p -values are indicated in italics.

	offspring inbreeding status		female size		interaction	
	F /LR χ^2	p -value	F /LR χ^2	p -value	F /LR χ^2	p -value
hatching success (%)	1.83	0.18	1.09	0.30	3.01	0.08
average larval mass (mg)	0.11	0.74	30.3	<0.001	1.05	0.31
survival to dispersal (%)	17.8	<0.0001	0.05	0.82	9.49	<0.01
survival to eclosion (%)	21.5	<0.0001	2.24	0.13	1.01	0.32
offspring adult lifespan (days)	24.9	<0.001	1.40	0.24	0.32	0.57

4. Discussion

In this study, we tested whether the mother's phenotype can influence the severity of inbreeding depression in her offspring. We found evidence for inbreeding depression in three of the five traits we measured: survival from hatching to dispersal, survival from dispersal to eclosion, and post-eclosion lifespan (table 1). In addition, we found a significant interaction between inbreeding and maternal body size on survival to dispersal. This interaction reflected that inbreeding depression in this trait was more severe for offspring of large females than offspring of small females (figure 2). There was no such interaction on survival to eclosion or post-eclosion lifespan. Although we found an interaction in only one of these fitness traits, we note that this trait was also the one with the highest level of inbreeding depression (figure 1). In summary, our key finding was that offspring of large females suffered a lower survival rate from hatching to dispersal if they were inbred than if they were outbred, whereas inbred and outbred offspring of small females had a similar survival rate (figure 2). To our knowledge, this is the first evidence for a causal effect of the maternal phenotype on the severity of inbreeding depression in the offspring.

Until now, very few studies have considered maternal effects in the context of inbreeding depression, and all of these were conducted on plants [38–41]. Moreover, none of these studies established a causal link between maternal effects and the magnitude of inbreeding depression. For example, Wolfe [38] found that maternal effects influenced early-life fitness traits in *Hydrophyllum appendiculatum*, while inbreeding depression affected late-life fitness traits. He proposed two plausible explanations for this pattern: (i) maternal effects substantially reduce the severity of inbreeding depression in early-life fitness traits, so no inbreeding depression is detected during this life stage or (ii) inbreeding depression is more severe in later life stages because of the cumulative effect of smaller fitness reductions in earlier life stages [38]. Nevertheless, Wolfe [38] could not distinguish between these two explanations, so it is unclear whether the mother's phenotype influences the severity of inbreeding depression in this species.

Here, we demonstrate that maternal body size can alter the severity of inbreeding depression in larval survival in the burying beetle *N. vespilloides*. Inbred offspring of large

females suffered lower survival from hatching to dispersal than outbred ones, whereas offspring of small females had the same survival rate regardless of their inbreeding status. The fact that there was no detectable inbreeding depression in this trait for offspring of small females suggests that maternal effects completely masked the deleterious effects of inbreeding on early-life offspring performance. In a population where the majority of females are small, such a masking effect could effectively hide the inbred genotype from natural selection, with potential consequences for the level of genetic load in the population [38].

We expected that inbreeding depression would be less severe for offspring of large females than those of small females, because females that are in better condition might have the capacity to provide more care. Yet, we found evidence for the opposite pattern. One plausible explanation is that large females have larger clutches (table 1), resulting in more intense sibling competition, which might in turn exacerbate the severity of inbreeding depression. We think this is unlikely given our earlier work showing that sibling competition does not exacerbate inbreeding depression in this species [22]. Additionally, our mean brood size was relatively small (potentially due to seasonal variation in reproductive success [42]), suggesting a low level of sibling competition in our study. An alternative explanation is that small and large females differ in how they invest in an inbred brood because of their different prospects for future breeding opportunities. Large females are more successful at acquiring and defending a carcass against conspecific competitors [30], so they have a higher residual reproductive value than small females, which may only breed once. Thus, a small female might maximize her reproductive effort during a breeding attempt regardless of her offspring's inbreeding status. On the other hand, when a large female mates with a relative and produces a brood of inbred, low-quality offspring, she might reduce her investment in current reproduction in order to take advantage of additional breeding opportunities in the future. Such adjustments in maternal investment could be mediated through changes in pre-hatching effort (e.g. egg size, nutrients deposited into the eggs) and/or post-hatching effort (e.g. provisioning rate), leading to a reduction in the survival of inbred offspring reared by large mothers. Nevertheless, this interpretation assumes that *N. vespilloides* females have the ability to recognize their relatives and/or the inbreeding status or overall quality

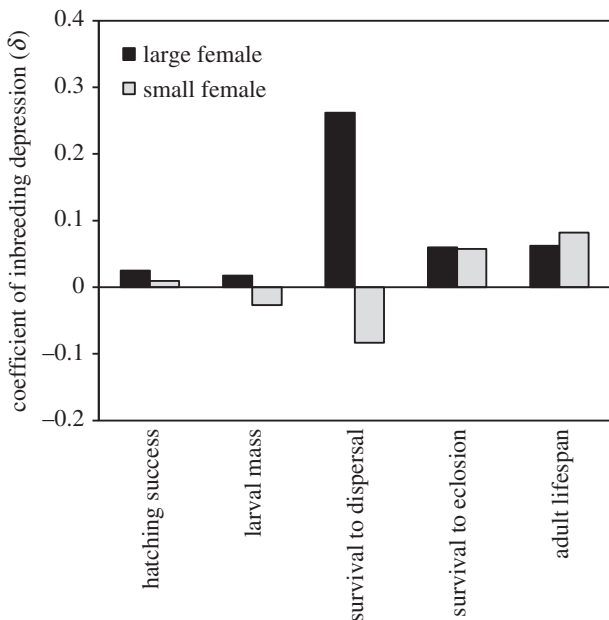


Figure 1. Inbreeding depression (δ) in offspring with large mothers (black bars) or small mothers (grey bars). Inbreeding depression was calculated as a proportional change in mean fitness of outbred (w_o) and inbred (w_i) offspring, using the equation $\delta = (w_o - w_i)/w_o$. Survival to dispersal is the offspring survival rate from hatching to dispersal, which corresponds to the end of the parental care period. Survival to eclosion is the offspring survival rate from dispersal to eclosion. Adult lifespan refers to the number of days an individual was alive after eclosion.

of their offspring. Further work is needed to determine whether females behave differently towards inbred and outbred offspring.

We expect inbreeding to be relatively rare in *N. vespilloides*, which makes this species a good model for understanding how the fitness costs of inbreeding are influenced by maternal effects in species that do not regularly inbreed. There are two important reasons it is useful to focus on species where inbreeding is relatively rare: (i) inbreeding depression is a greater concern for species with no prior history of inbreeding because deleterious, recessive alleles have not yet been purged from the population and (ii) once a species has a significant history of inbreeding, parental behaviours and other family interactions might be modified by selection due to inbreeding. Thus, species with a history of inbreeding might not be appropriate as models for endangered species that have only recently become subject to inbreeding. In principle, all populations are potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding [3]. It is therefore important to improve our understanding of how populations that become subject to inbreeding may cope with inbreeding depression.

Our study shows that maternal effects have the potential to influence the magnitude of inbreeding depression in the offspring. We encourage future research to investigate this previously unexplored issue in other taxa, because this pattern may be widespread in natural populations that suffer from inbreeding depression. If that is the case, it could have important implications for conservation efforts. Maternal effects are inextricably linked to maternal condition, which may vary over time within a population due to seasonal changes in weather and food availability [43–45]. Maternal effects may therefore contribute to temporal variation in

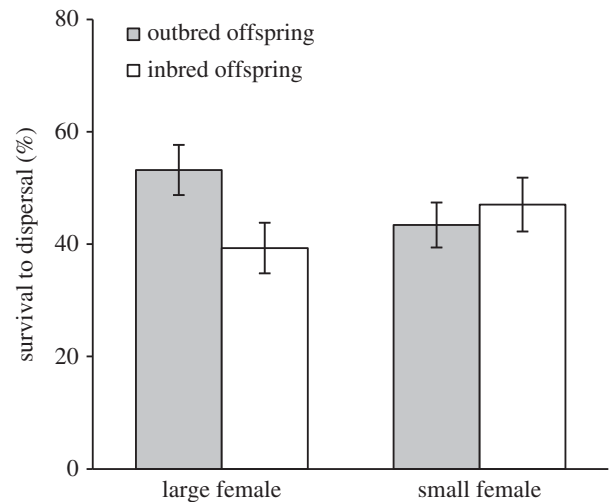


Figure 2. Mean (\pm standard error (s.e.)) survival rate from hatching to dispersal for outbred (grey) or inbred (white) offspring of small or large females. Inbred offspring of large females suffered lower survival from hatching to dispersal than outbred ones, whereas offspring of small females had the same survival rate regardless of their inbreeding status.

inbreeding depression in natural populations that are subject to environmental variability. In species where estimates of inbreeding depression (δ) are sensitive to maternal condition, a better understanding of the role of maternal effects may be important for the conservation and management of endangered populations. The presence of maternal effects might cause researchers to overestimate or underestimate inbreeding depression as a threat to population viability depending on the state of females at the time of data collection and on the particular subsample of females used in the study.

Our findings also have general implications for evolutionary biology, because if maternal condition influences inbreeding depression in the offspring, we might expect selection for inbreeding avoidance to depend on the average maternal condition in the population. Depending on the parents' capacity to moderate the deleterious effects of inbreeding in the offspring, there might be selection for inbreeding avoidance, tolerance or even preference. Existing theory [46–48] has overlooked the possibility that maternal effects might influence animal inbreeding strategies. Until now, theoretical models have focused on how the costs of dispersal, mating system, mate encounter rate, and kin recognition might shape the balance between inbreeding tolerance and avoidance [46–48]. We propose that incorporating maternal effects into such models may help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding, which has so far been challenging [2].

In summary, our study provides novel insights into the role of maternal effects in altering the expression of inbreeding depression. We show that inbreeding depression in larval survival was less severe for offspring of small females than for offspring of large females. This pattern might be driven by differences in how small and large females invest in an inbred (low-quality) brood because of their different prospects for future reproduction. We recommend that future research investigates how other maternal traits, such as age, nutritional condition, and health status, might affect the severity of inbreeding depression within or among populations. In natural populations that are subject to inbreeding, maternal effects may contribute to both variation in the magnitude of

inbreeding depression and variation in the strength and direction of selection for inbreeding avoidance.

Data accessibility. The raw data are available on the Dryad Digital Repository [49].

Authors' contributions. N.P. conceived the study, designed the study, collected data, carried out the statistical analyses, and wrote the manuscript. P.T.S. helped design the study and provided feedback on the manuscript.

Competing interests. We have no competing interests.

Funding. N.P. and P.T.S. were funded by the Institute of Evolutionary Biology and the School of Biological Sciences at The University of Edinburgh. N.P. was also supported by a Student Research Award from the American Society of Naturalists.

Acknowledgements. We thank the Edinburgh Countryside Rangers for permission to collect beetles at Corstorphine Hill. We are also grateful to Deborah Charlesworth, Emma Cunningham, Lucy Ford, Jacob Moorad, Matthieu Paquet, and Craig Walling for helpful discussions on the experimental design and data analysis. Lastly, we thank Hope Klug, Sandra Steiger, and one anonymous reviewer for helpful comments on the manuscript.

References

- Charlesworth D, Charlesworth B. 1987 Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**, 237–268. (doi:10.1146/annurev.es.18.110187.001321)
- Szulkin M, Stopher KV, Pemberton JM, Reid JM. 2013 Inbreeding avoidance, tolerance, or preference in animals? *Trends Ecol. Evol.* **28**, 20–211. (doi:10.1016/j.tree.2012.10.016)
- Andersen LW, Fog K, Damgaard C. 2004 Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proc. R. Soc. Lond. B* **271**, 1293–1302. (doi:10.1098/rspb.2004.2720)
- Keller I, Lurgiàdèr CR. 2003 Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proc. R. Soc. Lond. B* **270**, 417–423. (doi:10.1098/rspb.2002.2247)
- Keller LF, Waller DM. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241. (doi:10.1016/S0169-5347(02)02489-8)
- Crnokrak P, Roff D. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260–270. (doi:10.1038/sj.hdy.6885530)
- Armbruster P, Reed DH. 2005 Inbreeding depression in benign and stressful environment. *Heredity* **95**, 235–242. (doi:10.1038/sj.hdy.6800721)
- Fox CW, Reed DH. 2010 Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution* **65**, 246–258. (doi:10.1111/j.1558-5646.2010.01108.x)
- Meunier J, Kölliker M. 2013 Inbreeding depression in an insect with maternal care: influences of family interactions, life stage and offspring sex. *J. Evol. Biol.* **26**, 2209–2220. (doi:10.1111/jeb.12217)
- Reed DH, Fox CW, Enders LS, Kristensen TN. 2012 Inbreeding-stress interactions: evolutionary and conservation consequences. *Ann. N. Y. Acad. Sci.* **1256**, 33–48. (doi:10.1111/j.1749-6632.2012.06548.x)
- Avilés L, Bukowski TC. 2006 Group living and inbreeding depression in a subsocial spider. *Proc. R. Soc. B* **273**, 157–163. (doi:10.1098/rspb.2005.3308)
- Pilakouta N, Jamieson S, Moorad JA, Smiseth PT. 2015 Parental care buffers against inbreeding depression in burying beetles. *Proc. Natl Acad. Sci. USA* **112**, 8031–8035. (doi:10.1073/pnas.1500658112)
- Andersen R, Gaillard J-M, Linnell JDC, Duncan P. 2000 Factors affecting maternal care in an income breeder, the European roe deer. *J. Anim. Ecol.* **69**, 672–682. (doi:10.1046/j.1365-2656.2000.00425.x)
- Bales K, French JA, Dietz JM. 2002 Explaining variation in maternal care in a cooperatively breeding mammal. *Anim. Behav.* **63**, 453–461. (doi:10.1006/anbe.2001.1954)
- Wong JWY, Kölliker M. 2012 The effect of female condition on maternal care in the European earwig. *Ethology* **118**, 450–459. (doi:10.1111/j.1439-0310.2012.02030.x)
- Steiger S. 2013 Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proc. R. Soc. B* **280**, 20131225. (doi:10.1098/rspb.2013.1225)
- Pilakouta N, Richardson J, Smiseth PT. 2015 State-dependent cooperation in burying beetles: parents adjust their contribution towards care based on both their own and their partner's size. *J. Evol. Biol.* **28**, 1965–1974. (doi:10.1111/jeb.12712)
- Wolf JB, Wade MJ. 2009 What are maternal effects (and what are they not)? *Phil. Trans. R. Soc. B* **364**, 1107–1115. (doi:10.1098/rstb.2008.0238)
- Mousseau TA, Fox CW. 1998 The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403–407. (doi:10.1016/S0169-5347(98)01472-4)
- Smiseth PT, Dawson C, Varley E, Moore AJ. 2005 How do caring parents respond to mate loss? Differential response by males and females. *Anim. Behav.* **69**, 551–559. (doi:10.1016/j.anbehav.2004.06.004)
- Mattey SN, Strutt L, Smiseth PT. 2013 Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: offspring suffer fitness costs when either they or their parents are inbred. *J. Evol. Biol.* **26**, 843–853. (doi:10.1111/jeb.12102)
- Pilakouta N, Sieber D, Smiseth PT. 2016 Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. *J. Evol. Biol.* **29**, 704–710. (doi:10.1111/jeb.12816)
- Scott MP. 1998 The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**, 595–618. (doi:10.1146/annurev.ento.43.1.595)
- Eggert A-K. 1992 Alternative male mate-finding tactics in burying beetles. *Behav. Ecol.* **3**, 243–254. (doi:10.1093/beheco/3.3.243)
- Arce AN, Johnston PR, Smiseth PT, Rozen DE. 2012 Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *J. Evol. Biol.* **25**, 930–937. (doi:10.1111/j.1420-9101.2012.02486.x)
- Smiseth PT, Moore AJ. 2004 Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behav. Ecol.* **15**, 621–628. (doi:10.1093/beheco/arh053)
- Fetherston IA, Scott MP, Traniello JFA. 1994 Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. *Anim. Behav.* **47**, 777–785. (doi:10.1006/anbe.1994.1110)
- Eggert A-K, Reinking M, Müller JK. 1998 Parental care improves offspring survival and growth in burying beetles. *Anim. Behav.* **55**, 97–107. (doi:10.1006/anbe.1997.0588)
- Rauter CM, Moore AJ. 2004 Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Anim. Behav.* **68**, 695–702. (doi:10.1016/j.anbehav.2003.09.018)
- Bartlett J, Ashworth CM. 1988 Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behav. Ecol. Sociobiol.* **22**, 429–434. (doi:10.1007/BF00294981)
- Lock JE, Smiseth PT, Moore AJ. 2004 Selection, inheritance, and the evolution of parent-offspring interactions. *Am. Nat.* **164**, 13–24. (doi:10.1086/421444)
- Pilakouta N, Richardson J, Smiseth PT. 2016 If you eat, I eat: resolution of sexual conflict over feeding from a shared resource. *Anim. Behav.* **111**, 175–180. (doi:10.1016/j.anbehav.2015.10.016)
- Pilakouta N, Halford C, Rácz R, Smiseth PT. 2016 Effects of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *Am. Nat.* **188**, 319–328. (doi:10.1086/687392)
- Monteith KM, Andrews C, Smiseth PT. 2012 Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. *J. Evol. Biol.* **25**, 1815–1822. (doi:10.1111/j.1420-9101.2012.02567.x)
- Billman EJ, Creighton JC, Belk MC. 2014 Prior experience affects allocation to current reproduction

- in a burying beetle. *Behav. Ecol.* **25**, 813–818. (doi:10.1093/beheco/aru051)
36. Johnston MO, Schoen DJ. 1994 On the measurement of inbreeding depression. *Evolution* **48**, 1735–1741. (doi:10.2307/2410261)
37. Moorad JA, Wade MJ. 2005 A genetic interpretation of the variation in inbreeding depression. *Genetics* **170**, 1373–1384. (doi:10.1534/genetics.104.033373)
38. Wolfe LM. 1993 Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. *Evolution* **47**, 374–386. (doi:10.2307/2410058)
39. Montalvo AM. 1994 Inbreeding depression and maternal effects in *Aquilegia caerulea*, a partially selfing plant. *Evolution* **75**, 2395–2409. (doi:10.2307/1940893)
40. Helenurm K, Schaal BA. 1996 Genetic and maternal effects on offspring fitness in *Lupinus texensis* (Fabaceae). *Am. J. Bot.* **83**, 1596–1608. (doi:10.2307/2445836)
41. Picó FX, Ouborg NJ, van Groenendael JM. 2003 Fitness traits and dispersal ability in the herb *Tragopogon pratensis* (Asteraceae): decoupling the role of inbreeding depression and maternal effects. *Plant Biol.* **5**, 522–530. (doi:10.1055/s-2003-44788)
42. Andrews C, Kruuk LEB, Smiseth PT. 2016 Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. *Behav. Ecol.* <http://beheco.oxfordjournals.org/content/early/2016/08/21/beheco.arw129.full>.
43. Toigo C, Gaillard J-M, Van Laere G, Hewison M, Morellet N. 2006 How does environmental variation influence body mass, body size, and body condition? Roe deer as a case study. *Ecography* **29**, 301–308. (doi:10.1111/j.2006.0906-7590.04394.x)
44. Rodriguez-Hidalgo P, Gortazar C, Tortosa FS, Rodriguez-Vigal C, Fierro Y, Vicente J. 2010 Effects of density, climate, and supplemental forage on body mass and pregnancy rates of female red deer in Spain. *Oecologia* **164**, 389–398. (doi:10.1007/s00442-010-1663-8)
45. Mason THE, Apollonio M, Chirichella R, Willis SG, Stephens PA. 2014 Environmental change and long-term body mass declines in an alpine mammal. *Front. Zool.* **11**, 69. (doi:10.1186/s12983-014-0069-6)
46. Waser PM, Austad SN, Keane B. 1986 When should animals tolerate inbreeding? *Am. Nat.* **128**, 529–537. (doi:10.1086/284585)
47. Lehmann L, Perrin N. 2003 Inbreeding avoidance through kin recognition: choosy females boost male dispersal. *Am. Nat.* **162**, 638–652. (doi:10.1086/378823)
48. Kokko H, Ots I. 2006 When not to avoid inbreeding. *Evolution* **60**, 467–475. (doi:10.1111/j.0014-3820.2006.tb01128.x)
49. Pilakouta N, Smiseth P. 2016 Data from: Maternal effects alter the severity of inbreeding depression in the offspring. Dryad Digital Repository. <http://dx.doi.org/10.5061/dryad.r754h>.