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Effects of inbreeding on behavioural plasticity of parent–offspring interactions in a burying beetle

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Abstract

Inbreeding depression is defined as a fitness decline in progeny resulting from mating between related individuals, the severity of which may vary across environmental conditions. Such inbreeding-by-environment interactions might reflect that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions better, as shown in prior studies on developmental plasticity. Behavioural plasticity is more flexible than developmental plasticity because it is reversible and relatively quick, but little is known about its sensitivity to inbreeding. Here, we investigate effects of inbreeding on behavioural plasticity in the context of parent–offspring interactions in the burying beetle *Nicrophorus vespilloides*. Larvae increase begging with the level of hunger, and parents increase their level of care when brood sizes increase. Here, we find that inbreeding increased behavioural plasticity in larvae: inbred larvae reduced their time spent associating with a parent in response to the length of food deprivation more than outbred larvae. However, inbreeding had no effect on the behavioural plasticity of offspring begging or any parental behaviour. Overall, our results show that inbreeding can *increase* behavioural plasticity. We suggest that inbreeding-by-environment interactions might arise when inbreeding is associated with too little or too much plasticity in response to changing environmental conditions.

KEYWORDS

begging, inbreeding-by-environment interactions, *Nicrophorus vespilloides*, parental care, phenotypic plasticity

1 | INTRODUCTION

Inbreeding, or mating between related individuals, is a key issue in ecology and evolution because of its impact on the persistence of populations and on their ability to evolve in response to changing environments (Charlesworth, 2003; Keller & Waller, 2002). Inbreeding is often associated with a decline in fitness of any resulting progeny, a phenomenon known as inbreeding depression (Davenport, 1908; East, 1908). Inbreeding depression is caused by greater homozygosity associated with inbreeding, which reduces fitness by increasing the risk that rare, deleterious and recessive alleles are expressed and exposed to selection (dominance hypothesis; Davenport, 1908)

or by reducing any potential benefits due to heterozygote advantage (overdominance hypothesis; East, 1908). The severity of inbreeding depression can vary across environments (Armbruster & Reed, 2005; Cheptou & Donohue, 2011; Fox & Reed, 2011), and sources of environmental stress, such as intense intraspecific competition (Haag, Hottinger, Riek, & Ebert, 2002; Meagher, Penn, & Potts, 2000), extreme temperatures (Bijlsma, Bundgaard, & van Putten, 1999; Fox, Stillwell, Wallin, Curtis, & Reed, 2011), parasitic infection (Haag, Sakwinska, & Ebert, 2003) and nutrient deprivation (Auld & Henkel, 2014; Schou, Loeschcke, & Kristensen, 2015), are known to exacerbate inbreeding depression. However, little is known about the mechanisms for these inbreeding-by-environmental stress interactions (Reed, Fox, Enders, & Kristensen, 2012). Potentially, environmental stress might exacerbate inbreeding depression by increasing the intensity of selection acting against deleterious alleles (Laffafian, King, & Agrawal, 2010) or by increasing the amount of phenotypic variation induced by stress, and thereby fitness differences between inbred and outbred individuals (Waller, Dole, & Bersch, 2008). A plausible underlying mechanism is that inbreeding is associated with reduced phenotypic plasticity (Bijlsma & Loeschcke, 2012; Fowler & Whitlock, 1999; Reed et al., 2012; Reed, Lowe, Briscoe, & Frankham, 2003). This mechanism requires that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions than outbred ones.

There is good empirical evidence that inbreeding alters developmental plasticity. For example, inbreeding reduces the duration of developmental growth in response to changing temperatures in *Drosophila subobscura* (e.g. Maynard Smith, Clarke, & Hollingsworth, 1955) and the development of morphological defences in response to the presence of predators in the freshwater snail *Physa acuta* (e.g. Auld & Relyea, 2010). Inbreeding also reduces plasticity in life history traits, such as laying date in response to advancing spring temperatures in red-cockaded woodpeckers (Schiegg, Pasinelli, Walters, & Daniels, 2002) and brood size in response to changes in resource availability in the burying beetle *Nicrophorus vespilloides* (Richardson, Comin, & Smiseth, 2018). On the other hand, inbreeding increases plasticity in the development of wing shape in response to changing temperatures in *Drosophila melanogaster* (Schou, Kristensen, & Loeschcke, 2015). However, little is known about the effects of inbreeding on behavioural plasticity; that is, how an individual adjusts its behaviour in response to changing environmental conditions. Unlike developmental traits, behaviours can change relatively quickly in response to variation in the social and physical environment. These changes are also reversible, allowing an individual to match its behavioural phenotype rapidly to environmental changes that occur within its lifetimes (Candolin & Wong, 2012; Piersma & Drent, 2003; Snell-Rood, 2013). Behavioural plasticity is likely to be linked to an individual's reproductive success and survival given that many behaviours play a key role during mating (e.g., Rodríguez, Rebar, & Fowlere-Finn, 2013), parenting (e.g. Royle, Russell, & Wilson, 2014), foraging (e.g., Sol, Timmermans, & Lefebvre, 2002) and avoidance of predators or pathogens (e.g., Benard, 2004). Understanding the interplay between behavioural plasticity and inbreeding is now an important challenge given that anthropogenic environmental change is expected to cause a reduction in population sizes, thereby increasing the risk of inbreeding, and induce changes in environmental conditions, such as resources required for breeding due to advancing spring temperatures (Schiegg et al., 2002). Thus, there is now a need for studies that investigate the effects of inbreeding on behavioural plasticity.

We investigate the effects of inbreeding on behavioural plasticity, focusing on behaviours expressed in social interactions between individuals. We examine these behaviours because the social environment is usually highly variable and social interactions often involve highly plastic behaviours (Foster, 2013). This is because individuals often adjust their behaviour in response to characteristics of the conspecifics with which they interact, such as their behaviour, body size or state, as well as the number of individuals in the group or the population. For instance, individuals often adjust aggression to the competitive ability of competitors (Simmons, 1986), mating behaviour to the availability or quality of mating partners (Kokko & Rankin, 2006; Kvarnemo & Ahnesjo, 1996), and parental behaviour to the presence of and/or the amount of care provided by their partner (Johnstone & Hinde, 2006) or the offspring's begging behaviour (Kacelnik, Cotton, Stirling, & Wright, 1995). Furthermore, there is evidence that inbreeding affects social interactions (e.g. Richardson & Smiseth, 2017; Mattey, Richardson, Ratz, & Smiseth, 2018), suggesting that inbreeding impacts how individuals respond to variation in their social environment. Inbreeding might alter behavioural plasticity in social interactions if inbred individuals invest less in costly mechanisms required for adaptive behavioural plasticity (Dingemanse & Wolf, 2013; Snell-Rood, 2013). These might include the necessary sensory and cognitive systems to perceive variation in the social environment, process the relevant information and mount a plastic behavioural response (Auld, Agrawal, & Relyea, 2010; Coppens, De Boer, & Koolhaas, 2010; DeWitt, Sih, & Wilson, 1998; Mathot, Wright, Kempenaers, & Dingemanse, 2012). If so, we expect inbred individuals to adjust their behaviour to the social context (requiring high cognitive abilities; Humphrey, 1976) less well than outbred ones. Altogether, we might expect behaviours expressed in social interactions to be particularly sensitive to the effects of inbreeding due to the key role of behavioural plasticity in social interactions and the potential impact of inbreeding on the necessary sensory and cognitive systems of such behaviours.

In this study, we investigate whether inbreeding alters the behavioural plasticity of offspring and parental behaviours expressed in parent–offspring interactions in the burying beetle *Nicrophorus vespilloides*. We focus specifically on parent–offspring interactions because both offspring and parental behaviours are highly flexible (Kilner & Johnstone 1997; Smiseth, Wright, & Kölliker, 2008; Royle et al., 2014). Larvae beg to obtain food from their parents, and parent provisioning predigested food to larvae (Eggert, Reinking, & Müller, 1998; Smiseth, Darwell, & Moore, 2003). Larvae adjust begging behaviour to their hunger state (which reflects the amount of food provisioned by parents in the recent past), spending more time begging when subject to food deprivation (Smiseth

& Moore, 2004, 2007). This plasticity in larval begging behaviour is likely to be adaptive given that begging is associated with both fitness benefits and fitness costs (Andrews & Smiseth, 2013; Takata, Mitaka, Steiger, & Mori, 2019). Likewise, parents adjust their parental behaviour in response to brood size, providing more care towards larger broods (Ratz & Smiseth, 2018; Smiseth, Lennox, & Moore, 2007; Smiseth, Ward, & Moore, 2007). This plasticity in parental behaviour is also likely to be adaptive given that parents caring for larger broods incur a fitness cost from providing more care (Ratz & Smiseth, 2018). Thus, assuming that larval and parental responses are adaptive, any changes in plasticity in larval behaviour in response to food deprivation and parental behaviour in response to brood size are likely to have detrimental fitness consequences. In addition, previous work shows that inbreeding affects larval begging behaviour (Mattey et al., 2018; Mattey & Smiseth, 2015), and offspring inbreeding affects the amount of care provided by outbred parents (Mattey et al., 2018; Mattey, Strutt, & Smiseth, 2013; Ratz, Castel, & Smiseth, 2018). Thus, inbreeding alters trait values of behaviours involved in parent–offspring interactions.

Our aim was to test for effects of inbreeding on behavioural plasticity by focusing on the interactions between inbreeding status and larval and parental behaviours across two environmental gradients. In the first experiment, we manipulated the inbreeding status of larvae (inbred or outbred) and monitored larval responses to variable lengths of food deprivation. In the second experiment, we manipulated the inbreeding status of parents (inbred or outbred) and monitored parental responses to variable brood sizes. If inbreeding reduced the ability of individuals to respond to variation in their environment, we predicted an effect of the interaction between the inbreeding status of larvae and food deprivation on the amount of time spent begging and/or associating with the parent by larvae. Likewise, we predicted an effect of the interaction between the inbreeding status of the parent and brood size on time spent provisioning food and/or associating with the brood by parents.

2 | MATERIALS AND METHODS

2.1 | Origin and rearing of experimental beetles

We used beetles from the 7–9th generations of an outbred laboratory population descending from individuals collected in Corstorphine Hill, Edinburgh, UK. The population was maintained under 20°C and a 16:8 hr light:dark photoperiod. Nonbreeding adult beetles were kept in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and fed organic beef twice a week. We minimized inbreeding in our stock population by avoiding breeding between closely related individuals (defined as individuals sharing at least one common grandparent), by maintaining a large stock population comprised of 100–150 breeding pairs per generation (Mattey et al., 2018), and by supplementing the stock population annually with wild-caught beetles from our collection

site in Blackford Hill, Edinburgh, UK. We produced inbred individuals by pairing full-sibling beetles from the stock population in the previous generation (Mattey et al., 2018). Given the negligible level of inbreeding in our stock population (see Mattey et al., 2018), inbred and outbred individuals had a coefficient of inbreeding of $F \approx 0.25$ and 0, respectively, when referenced to the local wild population from our collection site.

2.2 | Larval behaviour

In our first experiment, we manipulated the inbreeding status of larvae and monitored their response to three different levels of food deprivation. We manipulated the inbreeding status of larvae by assembling experimental broods where all larvae in the brood were either outbred ($N = 26$) or inbred ($N = 28$). To this end, we set up pairs of virgin outbred parents at the start of the experiment by placing a male and a female in a large plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass weighing 20.1–25.0 g. We generated inbred offspring by mating females to their full-sibling brothers and produced outbred offspring by mating other females to unrelated males. On the day before we anticipated the eggs to hatch (i.e. two days after the onset of egglaying; Smiseth, Ward, & Moore, 2006), we moved females and their carcasses to new containers lined with fresh soil (the males were discarded; Figure 1a) while leaving their eggs behind in the old container. These separations were done so that we could allocate an experimental brood made up of 15 same-aged larvae of mixed maternal origin to each female (Smiseth, Lennox, et al., 2007). We standardized our brood sizes in order to avoid potential confounding effects due to variation in brood size and larval age on larval behaviour (Paquet & Smiseth, 2017; Smiseth et al., 2003; Smiseth, Lennox, et al., 2007). We only allocated experimental broods to each female once her own eggs had hatched because parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller & Eggert, 1990).

For each brood, we collected data on larval behaviour at three different lengths of food deprivation: 0, 90 and 180 min. To this end, we performed three consecutive 15-min observation sessions on each brood over a 195-min period, starting 24 hr (±15 min) after a given brood was placed on a carcass. We recorded larval behaviour away from the mouse carcasses using a dead female parent as a stimulus. We did so to ensure that larvae had no access to food during the experiment, which otherwise would have interfered with our food deprivation treatment. Using a dead female as a stimulus also allowed us to exclude any potential effect of variation in female behaviour on larval behaviour (Smiseth, Andrews, Brown, & Prentice, 2010; Smiseth & Parker, 2008), and larvae beg towards a dead female in a similar way as towards a live female (Smiseth et al., 2010; Smiseth & Parker, 2008). We used dead female parents that had bred and produced a brood to ensure that larvae perceive them as caring parents (Smiseth et al., 2010).

We killed females used as a stimulus approximately 1 hr before the start of each behavioural session by freezing them for 30 min and
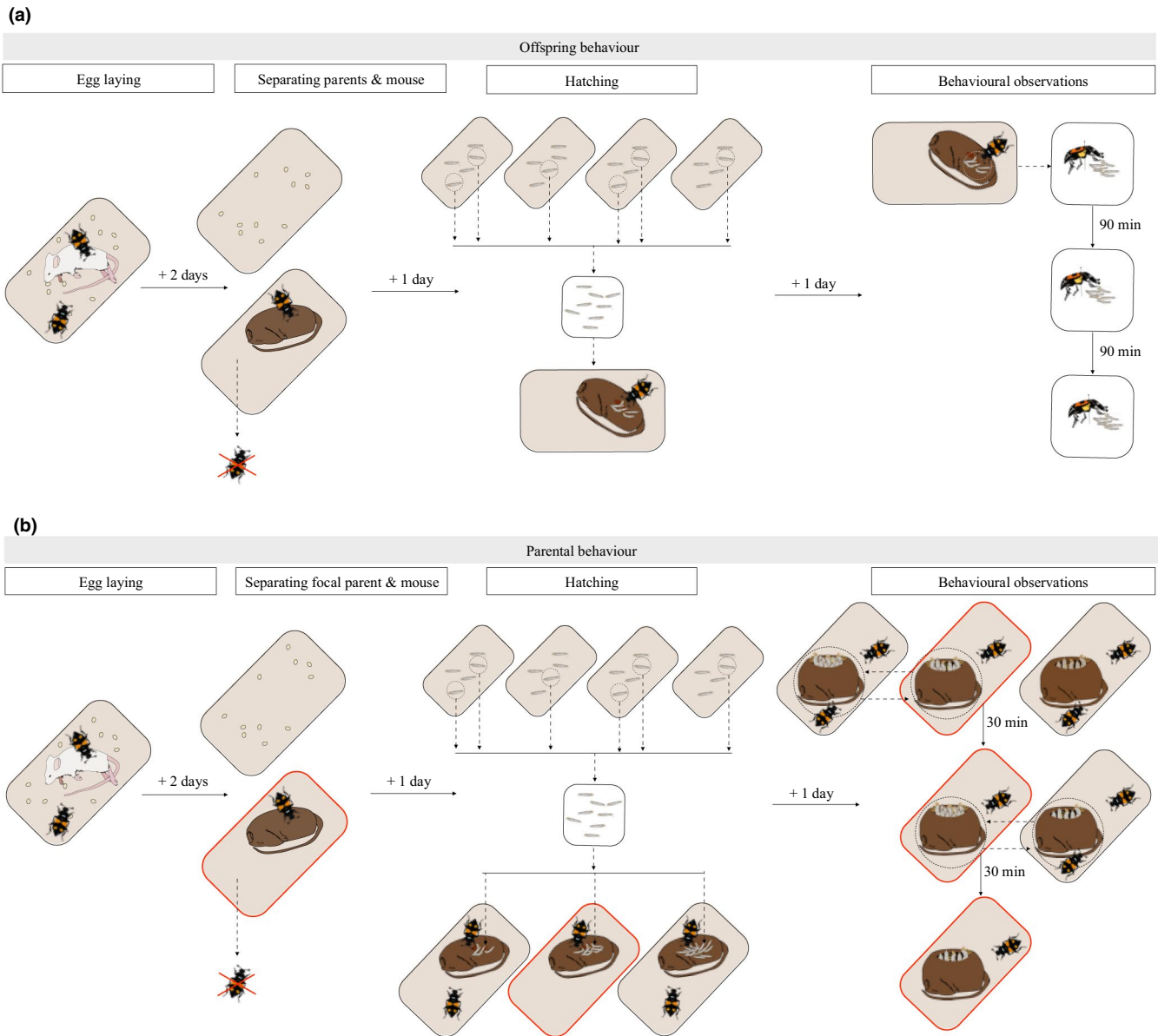
(a)

(b)

FIGURE 1 Diagram of the experimental design to investigate offspring response to the length of food deprivation (a) and parental response to variation in brood size (b)

then thawing them for another 30 min. Once thawed, we pinned each dead female to the centre of a small container (12 cm × 8 cm × 2 cm) lined with moist paper and in a position mimicking that of a parent provisioning food to the brood (Mäenpää, Andrews, Collette, Leigh, & Smiseth, 2015). We placed the experimental brood away from the female and left the larvae to acclimatize for 5 min before starting the first observation (see details below). Thus, in order to beg for food from the female, larvae first had to move towards the female to associate with her. Larvae might later move away from the female to search for other sources of food given that the female was dead and that larvae would receive no returns on their begging effort. Larvae were often observed to remain cohesive as a group, regardless of whether they were associating with the female or away from her. When away from the female, larvae would sometimes split into multiple groups and move around the container at a slow pace

either individually or in as a group. Note that each brood was placed with its caring female, and that larvae therefore always were exposed to a familiar female during the observation. After the first observation, the female was removed, and the larvae were kept in the container for another 75 min to give a total of 90 min of food deprivation. For the second observation, we again pinned the female in the centre of the container and returned the experimental brood to where it was placed at the start of the first observation. We repeated this procedure once more by removing the female at the end of the second observation and keeping the larvae in the container for another 75 min for a total of 180 min of food deprivation. Although larvae may not experience this level of food deprivation in natural situations, there will be natural variation in hunger level due to the time elapsed since they were last provisioned food by a parent (Smiseth et al., 2003). Larvae beg more and are hungrier when they

cannot receive food from a parent, suggesting that larvae are less efficient at obtaining food by self-feeding and have greater benefits when they obtain food from their parents (Smiseth & Moore, 2004). Furthermore, larvae may have limited access to those parts of the carcass that are most easily processed, especially when larvae are young and have relatively small mandibles (Eggert et al., 1998; Jarrett et al., 2018). We used our food deprivation treatments for pragmatic reasons, because it provides a straightforward procedure for generating variation in larval hunger levels (Smiseth & Moore, 2004, 2007).

During each observation session, we monitored larval behaviour every 60 s over a 15-min period. We recorded larval begging as the number of larvae that were touching any part of the female's body with their legs (Smiseth et al., 2003). We also recorded larval association with the female as the number of larvae that were within reaching distance from the female (i.e. a distance equal to or less than the pronotom length of the female). Based on these measures, we calculated the average time spent begging per larva in the brood (B) as the number of begging events cumulated across the 15 scans (Σb) divided by the cumulated number of larvae near the female (n), or $B = \Sigma b/n$. We also calculated the average time per larva in the brood spent associating with the female (A) as the number of larvae that were near the female across the 15 scans (Σa) divided by the total number of larvae in the brood (n), or $A = \Sigma a/n$.

2.3 | Parental behaviour

In our second experiment, we manipulated the inbreeding status of parents and monitored their response to small and large broods. In the previous generation, we generated inbred parents by mating their mother to her full-sibling brother, and we generated outbred parents by mating their mother to an unrelated male. We used both male and female parents in this experiment, allowing us to detect potential sex differences in behavioural plasticity of parents (Royle & Hopwood, 2017; Royle et al., 2014). Thus, we used a 2 × 2 factorial design in which we recorded the behaviour of 313 adult beetles. As we were interested in how parents adjust care in response to brood size, we excluded 175 individuals that were not observed providing care at least once to any one of the two broods. The final sample included 36 inbred males, 31 outbred males, 36 inbred females and 35 outbred females. To initiate breeding, we paired each experimental parent to an unrelated outbred partner. We placed the breeding pair into a larger plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass of a standardized size (20.3–23.9 g) (Livefoods Direct, Sheffield). We separated the parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil (Figure 1b). We discarded the partner at the same time to ensure that any effect of brood size on parental behaviour was not confounded by the presence of the partner. Once the eggs had started hatching, each experimental parent was allocated a brood of ten larvae (hereafter referred to as the baseline brood) to whom they provided care until being allocated the first experimental broods

24 hr later (see below). To avoid filial cannibalism, we allocated baseline broods to parents only once their own larvae had hatched.

In parallel with setting up the experimental parents, we set up additional pairs of unrelated males and females. We did this to produce additional larvae that were used to generate both baseline and experimental broods. The additional pairs also functioned as foster parents for the small and large experimental broods until they were allocated to an experimental parent 24 hr after it had been allocated its initial baseline brood. As described for the experimental parents above, we separated foster parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil. However, we left both foster parents with the broods to ensure that all experimental broods had encountered both a male and a female parent. Once eggs had started hatching, we allocated each foster pair either a small brood of five larvae or a large brood of 20 larvae, which fall well within the range of natural brood sizes for this species (Smiseth & Moore, 2002). We used these brood sizes because prior studies have shown that parents provide double the amount of time spent caring towards a brood of 20 compared to a brood of five larvae (Ratz & Smiseth, 2018; Smiseth, Lennox, et al., 2007).

For each parent, we collected data on their parental behaviour towards two different brood sizes: 5 and 20 larvae. We performed two consecutive 30-min observation sessions for each parent, starting 24 hr (±15 min) after the parent had been provided with the initial baseline brood. We randomized the order in which experimental parents were provided with broods of different sizes. We first removed the original mouse carcass containing the baseline brood of 10 larvae and immediately replaced it with a carcass from a foster pair containing an experimental brood of either 5 or 20 larvae. We allowed the larvae to settle for 30 min before starting the first observation. Immediately after the first observation was completed, we replaced this carcass with a carcass from a different foster pair containing an experimental brood of the opposite treatment (five larvae if the first experimental brood had 20 larvae and vice versa). We again allowed the larvae to settle 30 min before starting the second observation.

During each observation session, we monitored the behaviour of experimental parents every 60 s over a 30-min period. We recorded parental provisioning of food to the brood as a mouth-to-mouth contact between the parent and at least one larva. We also recorded parental association with the brood as the parent being present on the carcass or within the crypt (the depression in the soil immediately surrounding the carcass). We calculated the percentage of time spent provisioning food to the brood and associating with the brood as the total number of scans the parents were performing the behaviour of interest (i.e. 0–30) divided by the number of scans in the observation session (i.e. 30).

2.4 | Statistical analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team, 2019) with the packages *car* (Fox

TABLE 1 Effects of the level of food deprivation, inbreeding status and the interaction between food deprivation, inbreeding status and the interaction between food deprivation and inbreeding on average larval begging and average time spent associating with the female by the larvae. Effects of brood size, inbreeding status and the interaction between brood size and inbreeding status and the interaction between brood size and inbreeding on the time spent provisioning and associating with the brood by the carrying parent. Values are obtained from GLMMs

	Environmental variable				Inbreeding status (outbred versus. inbred)				Environment:Inbreeding			
	Estimate	SE	z-value	P	Estimate	SE	z-value	P	Estimate	SE	z-value	P
Food deprivation (0, 90 or 180 min)												
Offspring Begging	0.850	0.095	8.99	**<.0001**	0.031	0.298	0.103	.918	0.046	0.131	0.351	.725
Offspring association with the parent	−0.891	0.236	−3.99	**<.0001**	1.70	0.762	2.23	**.026**	−0.891	0.330	−2.70	**.007**
Brood size (5 or 20 larvae)												
Time spent provisioning	1.37	0.109	12.6	**<.0001**	0.032	0.295	1.07	.284	−0.215	0.148	−1.46	.146
Time spent associating with the brood	1.00	0.261	3.85	**.0001**	0.394	0.607	0.650	.516	−0.127	0.367	−0.346	.730

Note: Statistically significant *p* values (<.05) are shown in boldface.
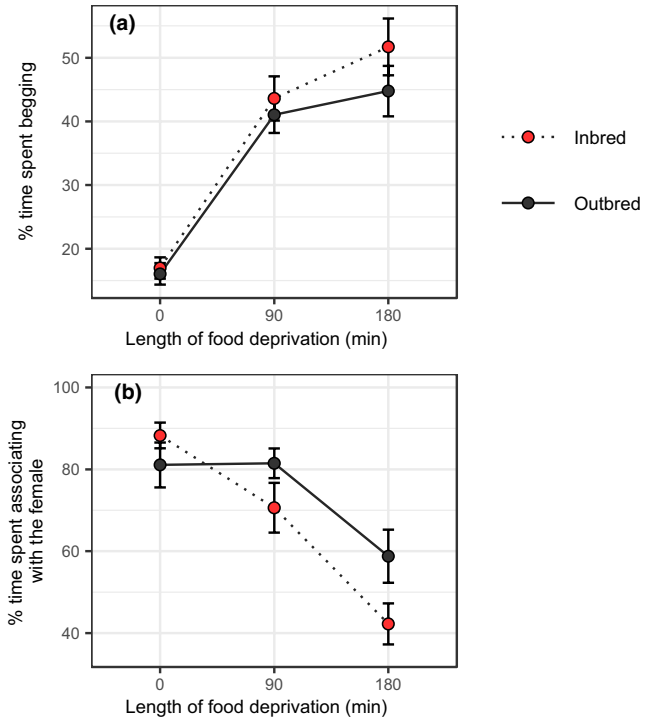

FIGURE 2 Effects of increasing length of food deprivation on the amount of time (percentage) larvae spent begging towards (a) and associating with (b) a female parent. Mean ± *SE*

et al., 2016) and *lme4* (Bates, Mächler, & Bolker, 2014). We quantified differences in behavioural plasticity between inbred and outbred larvae by estimating the effect of the interaction between the inbreeding status of larvae and the length of food deprivation on larval behaviour. We used general linear mixed models that assumed a binomial error structure to analyse larval behaviours (i.e. time spent begging towards and associating with the female). These models included the length of food deprivation (0, 90 and 180 min) as a continuous fixed effect and inbreeding status of larvae (inbred or outbred) as a categorical fixed effect, as well as the interaction between the two. We included brood size at the time of observation as covariate in the models to account for potential effects of brood size on larval behaviour. We also included brood ID and observation level as random effects to account for repeated observations on each brood and overdispersion of the data (Harrison, 2015), respectively.

To quantify differences in behavioural plasticity between inbred and outbred parents, we estimated the effect of the interaction between the inbreeding status of parents and brood size on parental behaviour. We used generalized linear mixed models that assumed a binomial error structure to analyse parental behaviours (i.e. time spent provisioning food and associating with the brood). These models included brood size (5 and 20 larvae) as a continuous fixed effect, inbreeding status of the parent (inbred or outbred) as a categorical fixed effect, and an effect of the interaction between the two. We also included sex of the parent as covariate to test for potential sex differences in the behavioural plasticity of parental behaviour. To account for repeated observations on the same focal individuals, we

included parental ID as random effects in both models. To account for overdispersion, we also included observation level as additional random effects in the model testing for effects on time spent associating with the brood.

3 | RESULTS

3.1 | Larval behaviour

Our main aim was to test for differences in behavioural plasticity between inbred and outbred individuals, and we therefore focused first on the interaction between the inbreeding status of larvae and the length of food deprivation on larval behaviour. There was no effect of this interaction on time spent begging (Table 1). Thus, for larval begging, there was no difference between inbred and outbred larvae with respect to behavioural plasticity in response to a change in hunger state (Figure 2a). However, there was a significant effect of this interaction on the amount of time spent associating with the female (Table 1), indicating that inbred larvae spent less time associating with the female as they became hungrier compared to outbred ones (Figure 2b). Thus, for time spent associating with the female, inbreeding increased behavioural plasticity exhibited by larvae in response to a change in their hunger state.

The length of food deprivation had a significant positive main effect on time spent begging and a negative main effect on time spent associating with the female (Figure 2a; Table 1). There was no main effect of the inbreeding status on time spent begging or associating with the female (Table 1). Finally, there was a negative main effect of brood size at the time of observation on time spent begging (estimate = −0.117, SE = 0.047, z = −2.47, p = .014), but brood size had no effect on time spent associating with the female (estimate = 0.012, SE = 0.120, z-value = 1.01, p = .314).

3.2 | Parental behaviour

For reasons explained above, we first focused on the interaction between the inbreeding status of the parent (inbred vs. outbred) and brood size (5 and 20 larvae) to test for potential differences in behavioural plasticity between inbred and outbred parents. There was no effect of this interaction on time spent provisioning food or associating with the brood (Figure 3a,b; Table 1). Thus, inbreeding did not appear to change behavioural plasticity exhibited by parents in response to changes in brood size.

As expected, brood size had a significant positive main effect on time spent provisioning food and associating with the brood (Table 1), confirming that parents spent more time provisioning food and associating with the brood when brood size increased. Finally, there were no main effects of parental inbreeding status (Table 1) or sex (estimate = −0.229, SE = 0.153, z-value = −1.50, p-value = .133) on time spent provisioning food. Likewise, there were no main effects of parental inbreeding status (Table 1) or sex (estimate = −0.255,
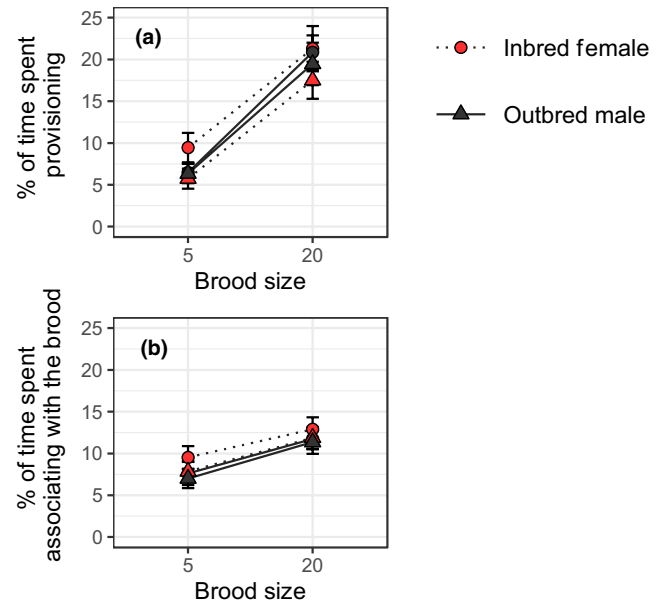

FIGURE 3 Effects of brood size on the amount of time (percentage) male and female parents spent provisioning food to (a) and associating with (b) the brood. Mean ± SE

SE = 0.244, z-value = −1.05, p-value = .296) on time spent associating with the brood.

4 | DISCUSSION

We show that inbreeding in larvae of *N. vespilloides* was associated with increased behavioural plasticity for time spent associating with the female parent. However, inbreeding was not associated with a change in behavioural plasticity in time spent begging or in the time that parents of either sex spent provisioning food or associating with the larvae. Our results derive from two experiments, in which we monitored behavioural plasticity in larvae in response to experimental variation in the length of food deprivation and behavioural plasticity in parents in response to experimental variation in brood size. We generated variation across two environmental stress gradients experimentally in order to remove confounding effects on plasticity in larval and parental behaviours. Furthermore, our study focused on behavioural plasticity in environmental gradients that larvae and parents are exposed to and respond to under natural conditions. Below, we discuss the wider implications of our results for our understanding of the effects of inbreeding on behavioural plasticity and how such effects may provide a mechanism for inbreeding-by-environment interactions affecting fitness.

Our study shows that larvae spent less time associating with the female as they became hungrier and that this decline was more pronounced in inbred larvae than in outbred ones. Currently, little is known about the potential adaptive value of behavioural plasticity in larval association with the female. Larvae associate with parents because they need to be in close proximity to them in order to beg for food (Smiseth & Moore, 2002). In our experimental design,

larvae had to move towards the female in order to be in close proximity to her. Larvae may later move away from the female because they would receive no returns on their begging given that we used a dead female as a standardized stimulus. Our results suggest that inbred and outbred larvae spent a similar amount of time associating with the female at the start of the experiment, but inbred larvae spent more time away from the female as the length of food deprivation increased (Figure 2b). Thus, our results show that inbred larvae had a greater degree of behavioural plasticity than outbred ones. Nevertheless, we urge caution when interpreting our results given that we monitored larval behaviour towards a dead parent in the absence of a carcass. We used a dead parent as a stimulus to ensure that larvae had no access to food during the experiment (which would otherwise interfere with our experimental treatment) and to control for confounding effects caused by parental behaviour (e.g. Smiseth et al., 2010; Smiseth & Parker, 2008). Prior work shows that the presence of a dead parent stimulates high levels of larval begging for at least 180 min (Smiseth & Parker, 2008). Yet, a consequence of this design is that larvae were exposed to an unresponsive parent for a considerable amount of time, which might explain why hungrier larvae spent *less* time associating with the female. In natural situations, where larvae interact with a live female on a carcass, we would expect hungrier larvae to spend more time associating with the female because larvae must stay in close proximity to her in order to have the opportunity to beg for food (Smiseth & Moore, 2002). In such situations, larvae face a choice between self-feeding from within the crater of the carcass (i.e. the cavity prepared by the parents) and leaving the crater to associate with a caring parent (Smiseth et al., 2003). Given that the larvae in our experiment could not get access to food from the dead female, and that there was no carcass from which to self-feed, larvae may have responded to food deprivation by associating less with the female and by searching for opportunities to obtain food by self-feeding (Smiseth et al., 2003). In light of this, we would not necessarily expect larvae to respond in a similar way to food deprivation when interacting with a live parent (Smiseth et al., 2003).

One potential explanation for our finding that inbreeding was associated with increased behavioural plasticity in larvae is that inbred larvae have higher nutritional needs than outbred ones. Offspring begging is thought to be an honest signal that reliably reflects the offspring's nutritional needs (Godfray, 1995), and there is good evidence that begging reflects larval hunger in our study species (Smiseth & Moore, 2004). Thus, if inbred larvae did have higher nutritional needs than outbred ones, we would expect inbred larvae to spend more time begging and to show greater plasticity in this behaviour. However, we found no evidence that this was the case as there was no effect of the interaction between larval inbreeding status and length of food deprivation on time spent begging. Furthermore, prior work on this species shows that inbred larvae spend *less* time begging to a live parent than outbred ones (Mattey et al., 2018). An alternative explanation is that inbred larvae were less able to sustain the costs of begging with an increase in the length of food deprivation than outbred ones.

This explanation, however, seems unlikely given that we found that inbred and outbred larvae increased their begging to similar degrees in response to an increase in the length of food deprivation. Thus, there is no evidence that our results can be explained as a consequence of inbred larvae having higher nutritional needs or greater costs of begging. A final explanation is that inbreeding constrains an individual's ability to invest in costly cognitive and/or sensory mechanisms required for adaptive behavioural plasticity (Dingemanse & Wolf, 2013; Snell-Rood, 2013). In this case, inbred individuals may not be able to adjust their behaviour as effectively to match changing conditions (e.g. Schiegg et al., 2002). For example, a recent study on our study species found that inbred females are less able than outbred females to adjust brood size when the size of the carcass is changed experimentally just prior to hatching (Richardson et al., 2018). Thus, inbreeding undermines the ability of burying beetles to make sensible life decisions, suggesting that our results may reflect that inbred larvae were less able to make an appropriate decision between staying near the female and searching for opportunities to self-feed.

Our finding that inbred larvae showed greater behavioural plasticity has important implications for our understanding of the mechanism for inbreeding-by-environment interactions. Inbreeding is often associated with an increased sensitivity to environmental stress (Armbruster & Reed, 2005; Cheptou & Donohue, 2011; Fox & Reed, 2011), and prior work suggests that such inbreeding-by-environment interactions may arise if inbreeding is associated with reduced phenotypic plasticity (Bijlsma & Loeschcke, 2012; Fowler & Whitlock, 1999; Reed et al., 2003, 2012). The rationale for this explanation is that inbred individuals are less able to adjust their phenotype to cope with stressful environmental conditions than outbred individuals. However, our results show that inbreeding can be associated with *increased* phenotypic plasticity. Increased behavioural plasticity may cause inbreeding-by-environment interactions for traits that are canalized because, for some traits, there may be selection that favours resistance to phenotypic plasticity (Schou, Kristensen, et al., 2015). For example, Schou, Kristensen, et al. (2015) found that inbred lines of *Drosophila melanogaster* had higher plasticity in the developmental response of wing size in response to high temperatures. This may come at a fitness cost as small wings may reduce flight performance in warmer environments more in inbred individuals (Frazier, Harrison, Kirkton, & Roberts, 2008). Just as there can be detrimental effects from too much developmental plasticity, stabilizing selection may also favour the evolution of intermediate levels of behavioural plasticity. Thus, if there is an optimal behavioural response, we might expect inbreeding-by-environment interactions if inbred individuals show either too much or too little behavioural plasticity. Furthermore, inbreeding-by-environment interactions could occur under stabilizing selection if inbred individuals show greater variance in behavioural plasticity, even if there is no difference in mean plasticity between inbred and outbred individuals. This would be the case if some inbred individuals show reduced behavioural plasticity whereas others show increased behavioural plasticity compared to outbred individuals. Thus, there is a need

for further work focusing on how selection works on behavioural plasticity.

In summary, we found that inbreeding affects behavioural plasticity of some larval behaviours (time spent associating with a parent), whereas inbreeding had no effect on behavioural plasticity of other larval behaviours (time spent begging) or any parental behaviours (time spent provisioning food and associating with the brood). To our knowledge, this is the first study investigating how inbreeding affects plasticity of social behaviours. Our findings suggest that effects of inbreeding on behavioural plasticity may be one of the potential mechanisms underlying the effects of inbreeding on social interactions among individuals (e.g. Richardson & Smiseth, 2017; Mattey et al., 2018). More generally, our findings have important implications for our knowledge about inbreeding depression by showing that inbred individuals can show *greater* behavioural plasticity in response to environmental variation than outbred ones. We suggest that effects of inbreeding on behavioural plasticity may cause inbreeding-by-environment interactions for traits where there are negative fitness consequences of showing either too much or too little plasticity in response to changing environmental conditions. We encourage more work on the interplay between inbreeding and adaptive behavioural plasticity given that inbreeding and stress due to environmental change are growing conservation concerns in many natural populations (e.g. Hamilton & Miller, 2016; Reed et al., 2012). Understanding the interplay between them will now be critical in our understanding of how natural populations respond to environmental change, such as climate change and population decline.

AUTHORS' CONTRIBUTION

T.R. and P.T.S. conceived and designed the experiment. T.R. and A.P. performed the experiment. T.R. analysed the data and wrote the first draft of the manuscript. All authors discussed the results and contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

Data deposited at dryad: https://doi.org/10.5061/dryad.c2fqz615b.

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