

Inbreeding depression increases with maternal age in a seed-feeding beetle

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ABSTRACT

Background: Interactions between inbreeding and maternal effects have received little attention, and the effect of maternal age on inbreeding depression in offspring has been almost entirely neglected. Maternal age affects allocation of resources and other materials to offspring, which can affect the fitness consequences of inbreeding. An interaction between inbreeding and maternal age thus has the potential to produce complex and long-lasting effects on population dynamics and evolutionary trajectories.

Hypothesis: Inbreeding depression in offspring fitness traits increases with maternal age at reproduction.

Organism: Two populations of the seed-feeding beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae).

Methods: We compared the effect of maternal age on egg development/hatch, larval survival, and larval egg-to-adult development time between offspring of sib-mated parents (offspring inbreeding coefficient: $F = 0.25$; parental $F = 0$) and offspring of outbred parents ($F = 0$ for both parents and offspring).

Results: The magnitude of inbreeding depression (proportional reduction in fitness of inbred relative to outbred beetles, δ) increased with maternal age for all measured traits (the proportion of eggs that developed, egg hatch, larval hatch-to-adult survival, and larval egg-to-adult development time). The age effect was large (δ increased by as much as 8% per day of maternal age), although the majority of this increase occurred only in the oldest age classes. There was no difference between the two beetle populations in the magnitude of this inbreeding–maternal age interaction.

Keywords: *Callosobruchus*, inbreeding depression, inbreeding–age interaction, inbreeding–environment interaction, maternal effects.

INTRODUCTION

Inbreeding depression results from increased genomic homozygosity. Increased homozygosity can occur at the level of offspring due to mating between parents that are more genetically similar than parents drawn at random from the population, or at the level of the

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population through loss of genetic diversity via random genetic drift (Charlesworth and Willis, 2009). The magnitude of inbreeding depression commonly varies with environmental conditions (Armbruster and Reed, 2005; Kristensen *et al.*, 2006, 2008; Nowak *et al.*, 2007; Reed *et al.*, 2007a, 2007b; Szulkin and Sheldon, 2007; Botham *et al.*, 2009), generally increasing with the magnitude of stress experienced during development (Fox and Reed, 2011). Inbreeding–environment interactions are important to a number of issues in evolutionary ecology and in conservation biology (Liao and Reed, 2009; Fox and Reed, 2011).

Despite the importance of inbreeding–environment interactions, studies examining environmental effects on inbreeding depression generally have either manipulated only a small diversity of commonly used stressors, such as temperature, diet or exposure to chemicals (see Table 1 in Fox and Reed, 2011), or compared inbreeding depression between periods of time among which fitness varies for unknown (only speculative) reasons [e.g. comparing inbreeding depression among years or seasons in natural populations (Reed *et al.*, 2007a, 2007b; Szulkin and Sheldon, 2007)]. The diversity of stressors that have been studied remains low compared with the diversity of ecological variables that affect fitness in plant or animal populations, limiting our ability to predict inbreeding depression in nature.

Maternal age has marked effects on offspring fitness in most animals. In general, older mothers produce less fit offspring (e.g. Richards and Meyers, 1980; Wasserman and Asami, 1985; Fox, 1993; Fox and Dingle, 1994; Hercus and Hoffman, 2000; Kern *et al.*, 2001), although there are many exceptions (Moore and Singer, 1983; Glazier, 1992; Berkeley *et al.*, 2004; Lock *et al.*, 2007). In insects, older mothers typically allocate fewer resources to eggs (e.g. Jones *et al.*, 1982; Wasserman and Asami, 1985; Boggs, 1986; Fox, 1993; Giron and Casas, 2003; Opit and Throne, 2007), and allocate different quality resources to eggs (e.g. Giron and Casas, 2003; Karl *et al.*, 2007). Offspring developing from these later-laid eggs are at a disadvantage relative to offspring from earlier-laid eggs; they generally take longer to develop to sexual maturity and have higher immature mortality (e.g. Richards and Meyers, 1980; Simmons, 1988; Rossiter, 1991; Wallin *et al.*, 1992; Fox, 1993; Fox and Dingle, 1994; Hercus and Hoffman, 2000). The change in allocation of resources from mothers to offspring thus increases developmental stress on offspring with increasing maternal age, which could mediate effects of inbreeding. However, despite these commonly large effects of maternal age on offspring fitness, it is unknown whether maternal age mediates, and potentially exacerbates, inbreeding depression.

Here we examine the interaction between inbreeding depression and maternal age in two populations of a seed-feeding beetle, *Callosobruchus maculatus*. In *C. maculatus*, older mothers produce substantially smaller eggs than do younger mothers and offspring hatching from these smaller eggs have a much increased risk of pre-maturation mortality, and those that survive to sexual maturity have increased development time (Wasserman and Asami, 1985; Fox, 1993). *Callosobruchus maculatus* also suffers substantial inbreeding depression throughout development; eggs produced from inbred matings are less likely to develop, have lower hatch rates, and larvae hatching from these eggs have ~20% lower hatch-to-adult survival compared with offspring produced from outbred matings (Fox *et al.*, 2007, 2008, 2011). Inbred offspring that survive to adults take ~5% longer to develop (Tran and Credland, 1995; Fox *et al.*, 2007) and have shorter adult lifespans (Fox *et al.*, 2006; Bilde *et al.*, 2009). This inbreeding depression increases with temperature and food stress (Fox and Reed, 2011). Here we extend these previous studies to examine whether inbreeding depression in offspring varies with maternal age, concordant with reduced maternal provisioning of older offspring compared with younger offspring.

MATERIALS AND METHODS

The life cycle of *C. maculatus* depends on seeds of its host plants. Females cement their eggs to the surface of host seeds (Messina, 1991). When eggs hatch, the first instar larvae burrow into the seed under the egg and larval development and pupation are completed within the single seed chosen by their mother.

We used two populations of beetles for this study. The South Indian (SI) population was collected in 1979 from infested pods of mung bean, *Vigna radiata* (L.) Wilczek, and the closely related black gram, *Vigna mungo* (L.) Hepper, from Tirunelveli, India (Mitchell, 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea, *V. unguiculata* (L.) Walp., from Ouagadougou, Burkina Faso (Messina, 1993). These two populations differ in body size, lifetime fecundity, patterns of egg dispersion, oviposition preference, and adult longevity (Fox *et al.*, 2004a, 2004b; Messina, 2004). Both populations were maintained in laboratory growth chambers on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at > 1000 adults per generation for > 200 (BF) or > 300 generations (SI) before this experiment.

We used a 'block design' to quantify inbreeding depression (Roff, 1998; see also Figure 1 in Fox *et al.*, 2011). Blocks were created by randomly pairing two families chosen from an outbred population. From each family, we randomly chose two females and two males to become parents. We crossed these two families, creating two inbred and two outbred pairs per block. The advantage of this design is that it ensures that inbred individuals are created from the same set of alleles as the outbred individuals to which they are to be compared (Fox, 2005). Matings were performed over a 10-day period but always at the same time of day (mid-afternoon).

Each mated pair was confined in a 35-mm Petri dish with ~40 seeds of mung (*V. radiata*) and allowed to lay eggs. Pairs were transferred to a new dish every 12 h for 7 days or until death. However, female egg laying declined substantially after 5.5 days, so only offspring produced during the first 5.5 days are used in this analysis. Eggs were maintained, and offspring were reared to adult, in a single reach-in growth chamber at 27°C and a light/dark cycle of 15:9. The proportion of eggs that developed, the proportion of developing eggs that hatched, and hatch-to-adult survivorship were scored for all eggs. Egg-to-adult development time was recorded for all surviving beetles except those hatching from eggs laid on the second day (half-day periods 1.5 and 2.0). Previous work has shown that there is at most a very small change in egg size, and no detectable change in larval survival, between eggs laid on the first versus the second day (Fox, 1993; Fox and Dingle, 1994).

In total, we scored development and hatch of 8087 eggs, and reared 3557 larvae to adults, from two inbred and two outbred families in each of 16 blocks (BF) and 20 blocks (SI).

Analyses

Blocks are the lowest level of independence in this design and thus block means were used in all analyses. All block means were calculated first by averaging across offspring within a family and then by averaging across families within the block and treatment. For survival data, each block contains two means for each age class (one mean each for inbred and outbred families). For development time, each block contains four means for age class, one for each sex × inbreeding treatment combination (inbred male offspring, outbred males, inbred females, and outbred females).

Inbreeding depression for survival was calculated as the proportional *reduction* in survival/mass:

$$\delta = \frac{\text{Mean}_{\text{outbred}} - \text{Mean}_{\text{inbred}}}{\text{Mean}_{\text{outbred}}}.$$

Inbreeding depression for development time was calculated as the proportional *increase* in development time:

$$\delta = \frac{\text{Mean}_{\text{inbred}} - \text{Mean}_{\text{outbred}}}{\text{Mean}_{\text{outbred}}}.$$

δ was calculated separately for each block, and each estimate of δ was treated as a single independent data point ($N = 16$ per age class for BF and $N = 20$ per age class for SI).

We used repeated-measures analysis of variance to test for variation in δ among maternal age classes, and then quantified the direction of the effect in two ways. First, we calculated the slope of the relationship between δ and maternal age for each block, and then averaged among blocks for an estimate of the average change in δ with age. However, most of the change in δ occurred only in the oldest age classes. We thus classified females as either 'young' (≤ 4 days old) or 'old' (> 4 days old) and tested for a difference in δ between age groups (one average estimate of δ per age category per block) using analysis of variance.

RESULTS

Detailed analyses of maternal age effects, and of population differences and environmental effects on inbreeding depression, are reported elsewhere (Fox *et al.*, 2007, 2011) and thus not presented here. We present only a brief overview of inbreeding depression and the pattern of maternal age effects for egg hatch, larval survival, and development time, and then focus more specifically on maternal age effects on inbreeding depression.

Inbreeding depression

Summed across all maternal ages, inbreeding depression was smallest for the proportion of eggs that developed ($\delta = 1\%$ and 2% for SI and BF population, respectively), larger for egg hatch (5% and 11%), and even larger for larval hatch-to-adult survival (23% and 31%). Overall, the proportion of eggs laid that gave rise to an adult offspring was 22% and 39% (SI and BF, respectively) lower for inbred than for outbred offspring, and development time of inbred offspring was 4% and 7% greater than development time for outbred offspring. These values for egg hatch, larval survival, and the proportion of eggs that give rise to an adult are slightly higher than those observed previously for these same two populations of *C. maculatus* in a study that included only young parents (Fox *et al.*, 2007).

Maternal age effects

The pattern of maternal age effects on outbred survival and development time are qualitatively the same, as described elsewhere (Wasserman and Asami, 1985; Fox, 1993; Fox and Dingle, 1994); the proportion of eggs developing, egg hatch, and hatch-to-adult survival all decreased and hatch-to-adult development time increased with increasing maternal age, with the largest changes being at the oldest maternal ages. We refer readers to analyses in previous

publications and to the Online Appendix (www.evolutionary-ecology.com/data/2623appendix.pdf) for details. More relevant to our specific interests here is that inbreeding depression varied with maternal age for all periods of larval development (Fig. 1): the proportion of eggs that developed (repeated-measures analysis of variance: $F_{10,322} = 2.71$, $P = 0.003$), egg hatch ($F_{10,317} = 2.69$, $P = 0.004$), and larval hatch-to-adult survival ($F_{8,180} = 2.08$, $P = 0.04$). There was no population difference in the variance in δ among maternal ages (population $\times$ maternal age interaction: $P > 0.09$ for all three traits). The magnitude of change in inbreeding depression with age was quite substantial for some traits (Table 1); for example, δ increased by as much as 0.07–0.08 per day of maternal age, which means that, on average, larval survival of inbred beetles through that particular period of mortality declined by 7–8%, relative to outbred larvae, per additional day of maternal age. However, most of this increase in δ occurred after females reached 4.0 days post-emergence. A comparison between young (≤ 4 days post-emergence) and old (> 4 days post-emergence) females indicated that δ was significantly greater for offspring of old females for all three periods of development ($P < 0.05$ for each). When excluding offspring from mothers > 4 days of age, there was no significant variance in δ with maternal age, suggesting that most of the age effect on δ is driven by the oldest age classes.

Inbreeding depression on larval development time also varied with maternal age (Fig. 2) (repeated-measures analysis of variance: $F_{8,144} = 3.32$, $P = 0.002$). However, this maternal age effect was driven entirely by a large increase in δ in the oldest maternal age class (age ≥ 5.5 days); after deleting this oldest age class (mothers ≥ 5.5 days) there was no detectable variation in δ among maternal age classes ($P = 0.10$).

DISCUSSION

In *Callosobruchus maculatus*, most components of fitness (e.g. egg-to-adult survival, development time) are lower for individuals hatching from eggs laid by older mothers. Similarly, inbreeding reduces all measured fitness components of *C. maculatus*. The novel result of our current study is that inbreeding and maternal age interact to affect an individual's fitness (i.e. the rate of offspring fitness decline with maternal age was significantly greater in inbred than outbred beetles). This inbreeding–maternal age

Table 1. Change in the magnitude of inbreeding depression, δ , per day ($\pm$ standard error)

Trait	Change in inbreeding depression (δ) per day of maternal age	
	South India population (SI)	Burkina Faso population (BF)
Eggs developing ^a	0.07 $\pm$ 0.03	0.04 $\pm$ 0.03
Eggs hatching ^b	0.04 $\pm$ 0.03	0.07 $\pm$ 0.03
Larval survival ^c	0.02 $\pm$ 0.03	0.08 $\pm$ 0.03

Note: These values are estimated by linear regression; we estimated the change in δ per day separately for each block, and then averaged across blocks.

^a The proportion of eggs producing a visible embryo. ^b The proportion of developing eggs that hatched (hatching was considered to have occurred if the larvae began digging into the seed). ^c The proportion of hatched eggs that produced an adult offspring that successfully emerged from the seed; offspring that pupated but failed to emerge from a seed were counted as part of larval mortality.

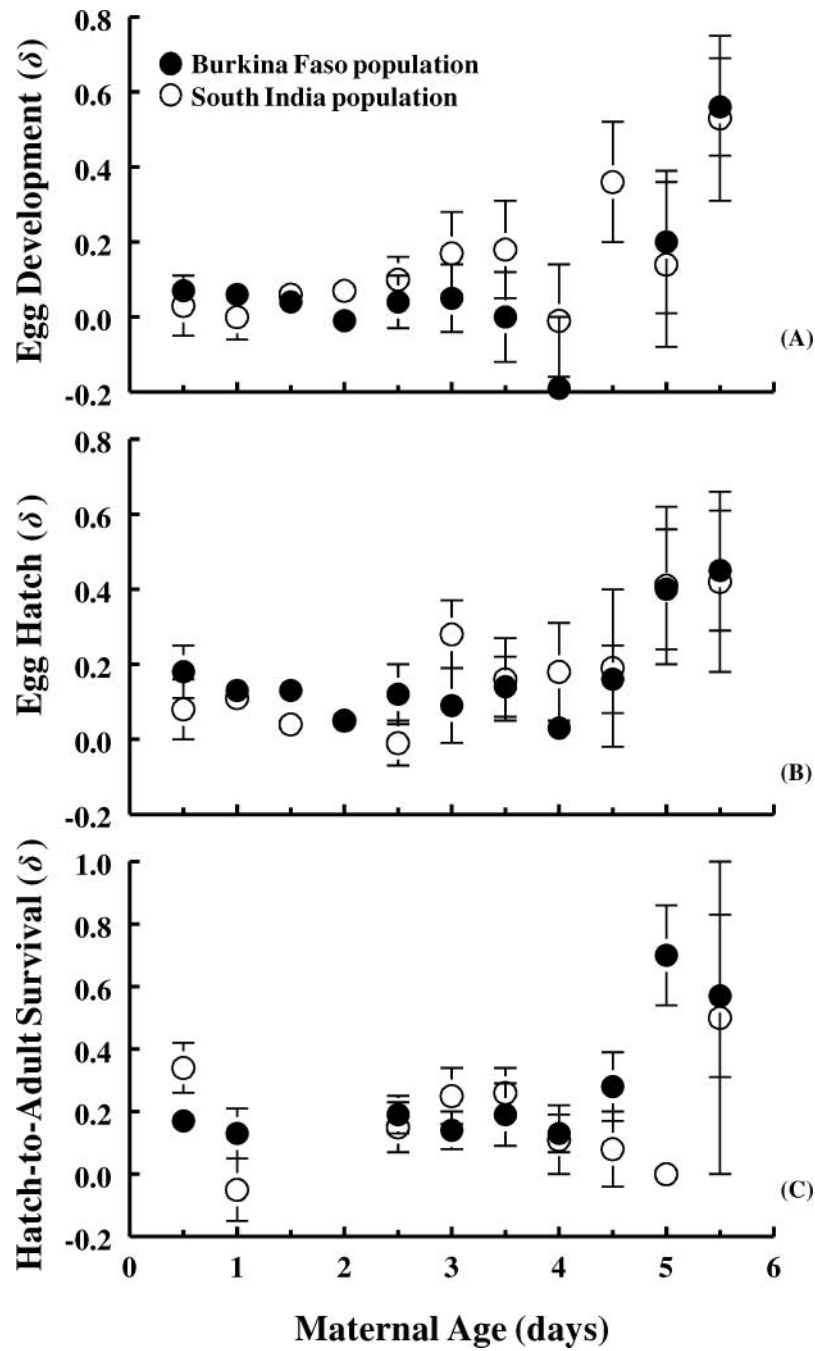


Fig. 1. The effect of maternal age on inbreeding depression (δ) for the proportion of eggs developing (A), the proportion of developed eggs hatching (B), and hatch-to-adult larval survival (C), in the seed-feeding beetle, *Callosobruchus maculatus*.

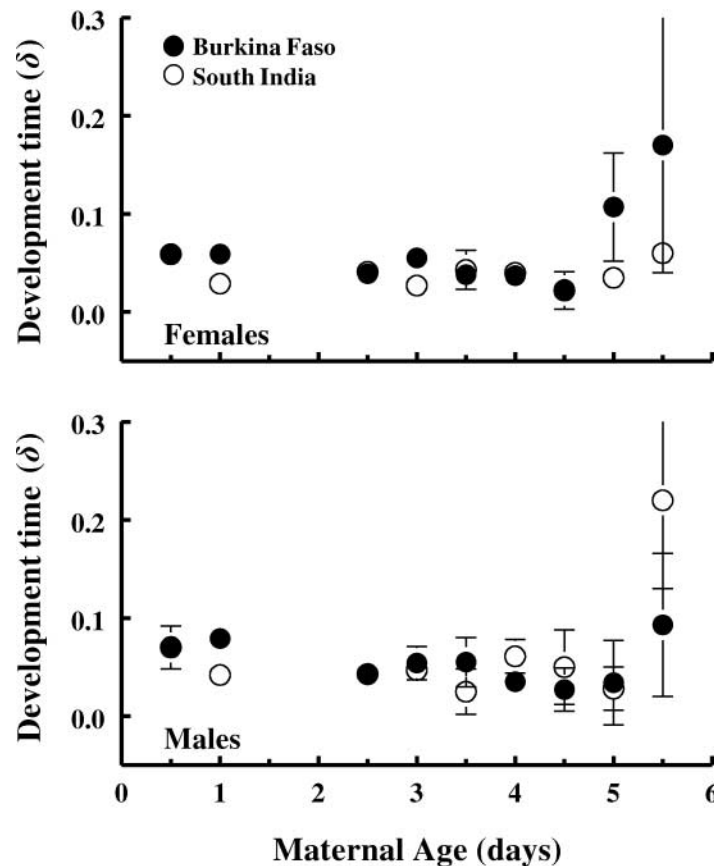


Fig. 2. The effect of maternal age on inbreeding depression (δ) for larval egg-to-adult development time of the seed-feeding beetle, *Callosobruchus maculatus*.

interaction is as predicted if increasing maternal age leads to decreasing resource provisioning of eggs, or increases in other kinds of stressors experienced by offspring during the early stages of development (Fox and Reed, 2011).

That maternal age affects offspring fitness traits (egg-to-adult survival and development time), as observed in this study, is commonplace in animals (e.g. Mousseau and Dingle, 1991; Fox, 1993; Fox and Dingle, 1994; Hercus and Hoffman, 2000; Kern *et al.*, 2001; Giron and Casas, 2003; Opit and Throne, 2007). In capital breeders, such as *C. maculatus*, these decreases in fitness covary with egg size (Fox, 1993), and can be mediated by maternal nutrient status (Fox and Dingle, 1994), and so are likely due, at least in part, to decreased quantity and/or quality of provisioning of offspring by older parents. In this study, viability at all stages of development declined with maternal age, as has been found in previous studies using outbred populations of seed-feeding beetles (Wasserman and Asami, 1985; Fox, 1993; Fox and Dingle, 1994). The decrease in offspring survival was not linear, but rather declined only slowly at early maternal ages but then dropped off quickly at older maternal ages.

The novelty of our study is in showing that this decline in offspring fitness with increasing maternal age was greater for inbred offspring than for outbred offspring. Both populations

of *C. maculatus* showed similar inbreeding–maternal age interactions for the proportion of eggs that developed, egg hatch, and larval hatch-to-adult survival. The inbreeding–maternal age interaction was strong, with the magnitude of inbreeding depression (δ) increasing by as much as an average of 0.08 per day of maternal age (for egg-to-adult survival). Just as with the direct maternal age effects on offspring survival, most of this increase in δ occurred in females more than 4 days of age. Inbreeding depression on larval development time also varied with maternal age, but this was almost entirely due to a large increase in δ in the oldest maternal age class.

Interactions between inbreeding and maternal effects have received little attention in the literature. Studies have demonstrated that effects of inbreeding are heritable by subsequent generations – that is, the inbreeding coefficient of parents affects the phenotype/fitness of their outbred offspring (Krebs and Hancock, 1990; Hauser and Loeschcke, 1995; Lyons, 1996; Hayes *et al.*, 2005), but studies of how maternal effects mediate inbreeding depression in offspring are largely lacking (but see Riginos *et al.*, 2007; Oh and Badyaev, 2008). In particular, the effect of maternal age on inbreeding depression in offspring has been neglected in the literature. Numerous studies have examined how the effects of inbreeding change with an *individual's* age, and a few studies have simultaneously examined the effects of maternal age and offspring inbreeding coefficient within a single study (e.g. Fuller and Strong, 1959; Cassinello, 1997; Urfer, 2009), especially in humans (Luna *et al.*, 2001, 2009). However, we know of only one other study, on humans (Yaqoob *et al.*, 1998), that has examined how inbreeding depression in offspring fitness traits changes with maternal age. A recent study on robins (Laws *et al.*, 2010) examined how the *maternal* inbreeding coefficient interacts with maternal age to affect offspring fitness traits, and found this maternal inbreeding–maternal age interaction to be the largest explanatory variable explaining offspring recruitment success. This is an interesting observation that, like our result, highlights the potential importance of an interaction between inbreeding and maternal age effects, but differs from our study in that we examined how inbreeding depression (due to variation in offspring inbreeding coefficient rather than maternal inbreeding coefficient) changes with maternal age.

Both ageing and inbreeding have been shown to be internal stresses that cause widespread and similar changes in gene expression levels (e.g. upregulation of Hsp70 expression), and that internal stresses can interact with external stresses to produce greater-than-additive decreases in fitness (Wheeler *et al.*, 1999; Landis *et al.*, 2004; Kristensen *et al.*, 2005, 2006; Pedersen *et al.*, 2005). However, in our study the stressor is not the age of the individual itself, but the age of the mother, and thus is necessarily a stressor that can be inherited by offspring. We propose that the increased stress on offspring with increasing maternal age in *C. maculatus* is due to changes in maternal provisioning of eggs. Thus, the interaction is potentially between an external stress (nutrient and/or caloric deprivation of hatchling larvae) and the internal stress caused by inbreeding. Resource stress should have particularly acute effects on inbreeding depression when that stress is experienced early in development, such that reduced maternal provisioning of eggs represents a particularly acute stress similar to food stress due to, for example, low prey availability for juvenile predatory insects and spiders (Reed *et al.*, 2007a, 2007b). To test the hypothesis that the inbreeding–maternal age interaction observed here is due to increased susceptibility of inbred larvae to resource stress, we need to perform diet manipulations, manipulating independently maternal and offspring diet and nutritional status.

Ramifications of inbreeding–age interactions

Maternal age represents a neglected source of variation in inbreeding depression. Maternal age affects a wide diversity of traits in probably all taxonomic groups, and thus has the potential to be an ecologically important influence on patterns of inbreeding depression in nature. For example, the interaction between maternal age and inbreeding level is important for the demographics and evolution of populations. Inbreeding is known to have multi-generational effects, with the offspring or even grand-offspring of inbred parents having lowered fitness, despite the fact that the descendents are not themselves inbred. Similarly, maternal effects are also known to last for multiple generations (e.g. Fox and Savalli, 1998; Hercus and Hoffmann, 2000). Thus, an interaction between inbreeding and maternal age has the potential to produce complex and long-lasting effects on population dynamics and evolutionary trajectories. Future studies, both empirical and theoretical, should consider how the interaction between inbreeding and maternal age affects important problems regarding such disparate topics as population viability, the evolution of mating systems, and the evolution of lifespan and ageing.

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