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Maternal effects on seed heteromorphism: a dual dynamic bet hedging strategy

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Short Communication

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Abstract

Maternal effects on offspring seeds are mainly caused by seed position on, and the abiotic environment of, the mother plant. Seed heteromorphism, a special form of position effect, is the production by an individual plant of morphologically distinct seed types, usually with different ecological behaviours. Seed heteromorphism is assumed to be a form of bet hedging and provides an ideal biological model to test theoretical predictions. Most studies of maternal effects on seeds have focused on abiotic environmental factors and changes in mean seed traits of offspring. *Suaeda salsa* is an annual halophyte that produces dimorphic seeds within the same inflorescence. We tested the hypothesis that plants grown from brown seeds of *S. salsa* have a higher offspring brown seed:black seed morph ratio and variance in seed size than plants from black seeds. Results from a pot experiment showed that plants grown from brown seeds had a higher brown seed:black seed ratio than plants grown from black seeds. This is the first layer of dynamic bet hedging. Brown seeds had higher size variation than black seeds, and seeds produced by plants from brown seeds also had higher seed size variation than plants grown from black seeds. This is the second layer of dynamic bet hedging. Thus, the maternal effect of seed heteromorphism is dual dynamic bet hedging. Furthermore, for seed traits we verified for the first time the theoretical prediction that an increase in offspring size variability induces an increase in the mean size of offspring.

Introduction

For plants, maternal effects occur when the abiotic environment experienced during growth and maturation of the maternal plant influences the offspring phenotype beyond the direct effect of transmitted genes (Gutterman and Fenner, 2000; Marshall and Uller, 2007). Seeds are simultaneously an important maternal component and subsequent offspring. Maternal conditions influencing seed traits include seed position on the parent plant (e.g. seed position in the fruit, seed position in the inflorescence, position of the inflorescence on the mother plant, etc.), age of the mother plant and abiotic environment of the plant (e.g. light quality, mineral nutrition or salinity) during seed development (Gutterman and Fenner, 2000; El-Keblawy *et al.*, 2016; Doudová *et al.*, 2017). Seed position can affect a series of seed traits in many plants, and it is often accompanied by seed heteromorphism (Gutterman and Fenner, 2000; Imbert, 2002; Kołodziejek, 2017).

Seed heteromorphism is the production by an individual plant of morphologically distinct seed types (morphs), usually with different ecological behaviours, including dispersal ability, dormancy and germination (Venable, 1985; Khan *et al.*, 2001; Imbert, 2002; Yao *et al.*, 2010; Liu *et al.*, 2018). For example, on one individual of the annual *Aethionema arabicum* mucilaginous seeds in dehiscent fruits support anti-telechory, while non-mucilaginous seeds in indehiscent fruits support telechory (Arshad *et al.*, 2019). Considering distinct seed traits, seed heteromorphism is an ideal biological phenomenon with which to test theoretical predictions about ecological adaptations.

Seed heteromorphism often is considered to be a typical example of adaptive bet hedging in a highly variable environment (Hughes, 2018). According to the model for the ecology and evolution of seed heteromorphism developed by Venable (1985), there are two kinds of seed heteromorphism: high-risk–low-risk (one morph with a high mean fitness but high risk of reproductive failure and another morph with a low mean fitness and a low risk of failure) and high-risk–high-risk (high mean fitness and high risk of reproductive failure for each seed morph). Production of two or more seed types with different ecological strategies by a single individual is adaptive via bet hedging in an unpredictable environment because among-generation variance of reproductive success is reduced, and thus the geometric mean fitness is

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increased (Crean and Marshall, 2009; Sadeh *et al.*, 2009; Simons, 2011; Hughes, 2018). Variation in seed morph ratio reflects the prediction of plants to good/bad years and the factors that influence this variation include the phenotype of maternal plant, nutrient availability, competition and others (Venable, 1985; Wang *et al.*, 2012; Doudová, 2017). Plants grown from heteromorphic seeds differ in biomass, salt tolerance, competition ability and seed production (Imbert, 2002; Wang *et al.*, 2012; Sendek *et al.*, 2015).

Most studies of maternal effects on seeds have focused on abiotic environmental factors or on changes in the mean offspring seed traits (Cheplick and Quinn, 1983; Galloway, 2005; Lu *et al.*, 2012). In fact, the phenotype of the mother plant can influence variation of offspring seed size (Wang *et al.*, 2012). Furthermore, there is size variation even within each seed type in heteromorphic plants (Mandák and Pyšek, 1999). Seed size variance is the response of mother plants to environmental unpredictability to gain maximum fitness (Wang *et al.*, 2012). *Suaeda salsa* (L.) Pall. (Amaranthaceae) is an annual halophyte, producing dimorphic seeds at specific positions in the inflorescence. Freshly matured brown seeds absorb water more quickly and have a higher germination percentage and velocity than black seeds under different levels of salinity (Song and Wang, 2015). Maternal salinity level can affect offspring seed germination and other traits (Wang *et al.*, 2015). Based on the theory of Venable (1985) and Hughes (2018), dimorphic seeds of this species show high-risk–low-risk heteromorphism: brown seeds represent the high-risk strategy and black seeds the low-risk strategy. Dynamic bet hedging is a phenomenon in which mother plants adaptively change the variation of offspring traits (Crean and Marshall, 2009). In this study, we tested the dual dynamic bet hedging hypothesis that plants grown from brown seeds of *S. salsa* have a higher offspring brown seed:black seed morph ratio and variance in seed size than plants from black seeds under different levels of salinity.

Based on the possibility that an offspring size fitness function is asymmetrical, Crean and Marshall (2009) speculated that an increase in offspring size variability will increase the mean size of offspring. In this study, we tested this hypothesis using dimorphic seeds of *S. salsa*.

Materials and methods

Study species and seed collection

Suaeda salsa, a succulent halophyte, occurs on saline soil or in inter-tidal zones. The average total salt content of dry soil at the sampling site is 5.2 g kg^{-1}. The average annual temperature is 13°C and the annual rainfall is 560–690 mm. Seeds germinate in April, and plants mature seeds in October in the field. Freshly matured fruits of *S. salsa* were collected from plants in a natural population (37°20′N; 118°36′E) growing in saline soil on the Yellow River Delta in Shandong Province, China, in October 2011. Fruits were taken from at least 50 plants and allowed to dry naturally at ambient room conditions. Seeds were separated and sorted into brown and black seeds. The mean length of brown and black seeds was 1.38 and 1.14 mm, respectively. The brown seed:black seed ratio was 2.4. The dimorphic seeds were stored at 4°C until transferred to the Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, in March 2012. The seeds were then stored at room temperature until used in this study.

Pot experiment

A pot experiment was carried out in a greenhouse (28 ± 3°C by day, 18 ± 3°C at night, natural light conditions) at Fukang Field Research Station of the Chinese Academy of Sciences (44°17′N, 87°56′E; 460 m above sea-level) in Xinjiang Province, China. To reduce the effect of differential germination time of the dimorphic seeds of *S. salsa*, black seeds were cold stratified for 20 days to break dormancy. About 20 seeds of each morph were sown at a soil depth of 2 mm in individual plastic pots (17 cm deep and 16 cm in diameter) filled with 2000 cm^3 mix of quartz sand and vermiculite (3:1 v/v) on 15 April 2012. After 20 days, seedlings were thinned to one per pot, at which time all seedlings were about 3 cm tall.

A randomized block design with six replicates was used. Each block contained six pots representing a combination of two seed morphs and three salinity levels. There was a total of 36 pots. For fertilization, a commonly available granular lawn fertilizer (Osmocote 301, Scotts, Marysville, OH, USA) and a commercial nutrient solution (Peters1, Scotts) were used. Each pot received 1.2 g Osmocote 301 once before sowing as basic fertilizer and 100 ml Peters1 nutrient solution (0.03 g l^{-1}) once each week as supplemental fertilizer, beginning 4 weeks after sowing. For salinity treatment, a mixed salt with a 20 $NaCl$:20Na_2SO_4:1$NaHCO_3$ (mass ratio) was used. The pots receiving low, moderate and high salinity received 100 ml salinity solution (0.3 g l^{-1}) twice a week, 100 ml salinity solution (1.1 g l^{-1}) twice a week and 100 ml salinity solution (4.4 g l^{-1}) twice a week, respectively. The pots were watered every day. The experiment was terminated on 25 October 2012, and offspring seeds were the harvested. This is a self-compatible and autogamous (or wind-pollinated) species (Jie Song, Shandong Normal University, personal communication, 1 March 2019).

Seed production and size

The mass of each seed morph for each individual plant was determined using a microbalance (Chyo Balance, JPN-200W). Offspring seed ratio was calculated as the mass of brown seeds divided by the mass of black seeds on a single plant. The diameter of 20 randomly selected brown and black seeds from each plant was measured using a computer imaging system. Seed size variation was expressed as the coefficient of variation per treatment: CV = (standard deviation/mean) × 100%.

Statistical analyses

ANOVA assumptions were checked, and seed ratio data followed a normal distribution. Two-way ANOVA was used to determine the significant effects of salinity and maternal seed type and their interaction on offspring seed ratio. Data for seed diameter and its CV were analysed by linear regression using the enter linear regression method (all independent variables ware entered into the equation in a single step). The multiple linear regression model included maternal seed type, offspring seed type (brown seed and black seed) and salinity (low, moderate and high). All statistical analyses were conducted using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

We counted the block effects, and there was no significant effect ($P = 0.136$). ANOVA results showed that the offspring seed ratio

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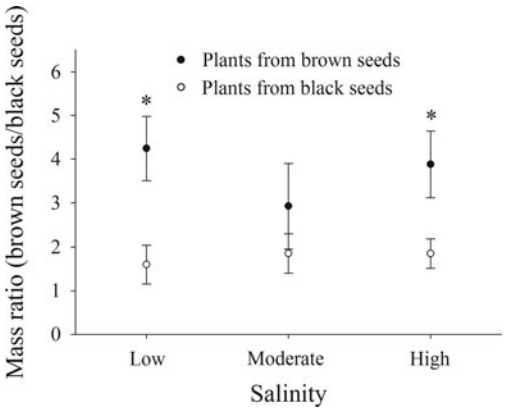


Fig. 1. Effect of maternal seed type on the brown seed:black seed mass ratio of *S. salsa* plants grown at three levels of salinity. *Significant difference between the two plant types at a salinity level with Tukey's test ($P < 0.05$). Error bars represent standard error (SE).

was significantly affected by maternal seed type ($P = 0.01$), but it was not significantly affected by salinity ($P = 0.455$) or the interaction between salinity and seed morph type ($P = 0.834$). Seed ratio (mean values ranged from 2.93 to 4.24) of plants from brown seeds was higher than that (from 1.60 to 1.85) of plants from black seeds (Fig. 1).

Offspring seed diameter was significantly affected by maternal seed type ($P < 0.001$) but not significantly affected by offspring seed type ($P = 0.190$) or salinity ($P = 0.697$) (Table 1). For each seed type, seed diameter of plants from brown seeds was significantly higher than that of plants from black seeds under different levels of salinity (Fig. 2a,b).

Variability in diameter of offspring seeds was significantly affected by maternal seed type ($P < 0.05$), offspring seed type ($P < 0.001$) and salinity ($P < 0.05$) (Table 1). The CV of brown seed diameter was significantly higher than that of black seeds (Fig. 2c,d). Seed size variability of plants from brown seeds was higher than that of plants from black seeds (Fig. 2c,d). With an increase in maternal salinity, variation in offspring (seed) diameter of both seed types in plants from brown seeds decreased accordingly. However, for mother plants from black seeds variation in diameter of dimorphic seeds was lowest at moderate salinity (Fig. 2c,d).

Discussion

Effects of the maternal seed type on offspring seed traits have been studied extensively (Imbert, 2002; Lu *et al.*, 2012; Wang *et al.*, 2012; Doudová *et al.*, 2017). However, our data are the first to document variance in offspring seed size via interactions between maternal seed type and offspring seed type. In addition, our data indicate that plants grown from brown seeds of *S. salsa* have a higher offspring brown seed:black seed ratio and variance in seed size than plants from black seeds. The results suggest that *S. salsa* has a dual dynamic bet hedging strategy, which would be adaptive to the environmental uncertainty of its saline habitat (Song and Wang, 2015), but to show this fitness needs to be determined.

Our results indicated that regardless of the maternal seed type, mother plants produce both types of seeds. This result suggests that both plant types have a bet hedging strategy because they cannot successfully predict their offspring's environment.

Table 1. Multivariate analysis of correlates associated with diameter and its CV of heteromorphic seeds of *S. salsa*

		B (SE)	β	*P*-value
Diameter	Salinity	−2.365 (6.075)	−0.010	0.697
	Offspring seed type	13.003 (9.920)	0.033	0.190
	Maternal seed type	−129.930 (9.920)	−0.326	<0.001
CV of diameter	Salinity	−0.717 (0.331)	−0.176	0.034
	Offspring seed type	−4.633 (0.540)	−0.696	<0.001
	Maternal seed type	−1.305 (0.540)	−0.196	0.018

SE, standard error.

However, plants grown from brown seeds produced a higher brown seed:black seed ratio than those produced by plants from black seeds. Brown seeds of *S. salsa* are non-dormant and have high germinability, high salt tolerance and a high-risk strategy, whereas black seeds are dormant and have low germinability, low salt tolerance and a low-risk strategy (Venable, 1985; Song and Wang, 2015). The function of producing dimorphic seeds is to minimize total fitness variance. The function of producing more brown seeds by plants grown from brown seeds is to maximize the fitness-risk ratio (Hughes, 2018). Thus, the ratio of dimorphic seeds is a risk index, and plants from brown seeds have a higher risk index than those from black seeds. This seed-set pattern suggests that mother plants can adaptively adjust variance by changing the seed type ratio, i.e. a form of dynamic bet hedging, which Sadeh *et al.* (2009) called plastic bet hedging.

Not only did the ratio of dimorphic seeds vary but seed size also varied. Size variation of brown seeds of *S. salsa* was significantly higher than that of black seeds. Furthermore, seeds produced by plants from brown seeds had a higher size variation than those produced by plants from black seeds. Maternal effect of seed size variation suggests that mother plants adaptively adjust seed traits above the effect of the dynamic shift in seed morph ratio, which is recognized as the second layer of dynamic bet hedging. Thus, the maternal effect of seed heteromorphism is probably dual dynamic bet hedging. There are some important ecological implications of this dual strategy. On the one hand, *S. salsa* produces heteromorphic seeds and increases within-clutch variation in seed size that allows the species to cope with environmental uncertainty. On the other hand, it adaptively adjusts seed morph ratio and seed size of each morph according to the environmental predictability of mother plants. This integrated strategy might be a trade-off between geometric mean fitness among generations and arithmetic mean fitness within generations (Crean and Marshall, 2009).

In our experiment, different salinity treatments did not significantly affect seed morph ratio and most variation of seed size. Similar findings have been reported for *Suaeda aralocaspica* (Wang *et al.*, 2012). This result can be explained by the fact that our salinity levels just reflect the range of salinity in the field and not the range of salinities that limit plant growth and reproduction of this species. The brown seed:black seeds ratio of *S. salsa* plants grown from brown seeds is 3.8 under 500 mmol l^{-1} NaCl (Wang *et al.*, 2015). This salinity treatment is in the range between moderate and high salinity treatment in

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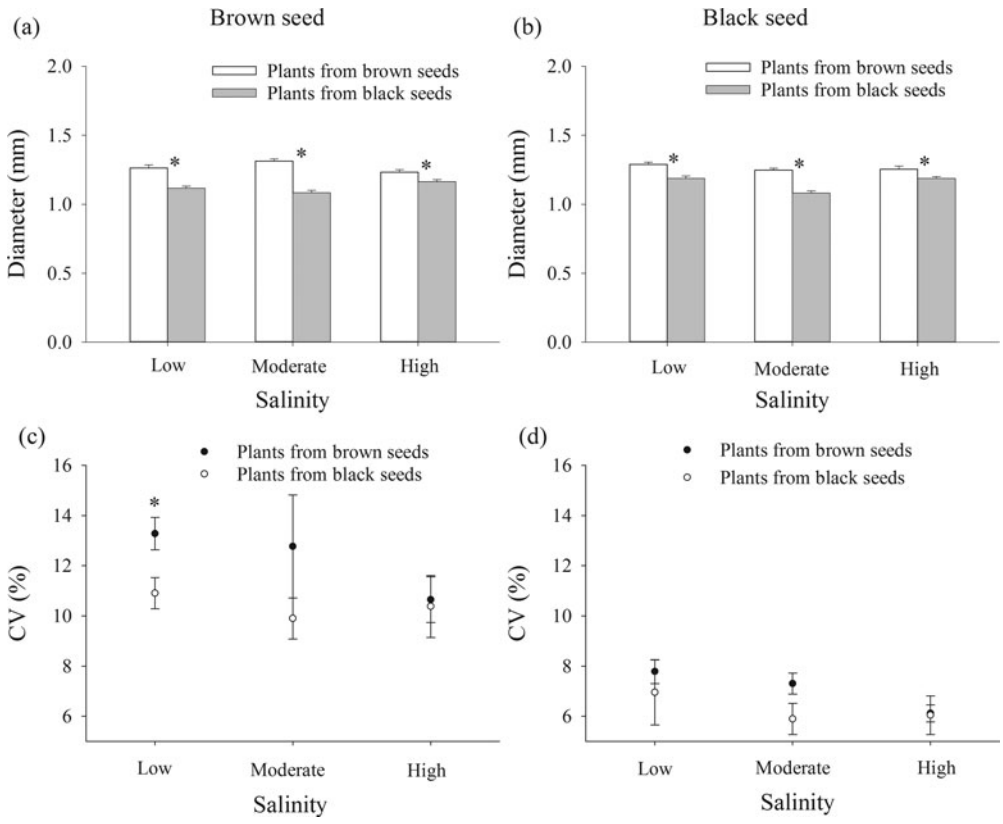


Fig. 2. Effect of maternal seed type on mean diameter of (a) brown and (b) black seeds, and on CV (coefficient of variation) of seed diameter for (c) brown and (d) black seeds of *S. salsa* grown at three levels of salinity. *Significant difference between the two plant types at a salinity level with Tukey's test ($P < 0.05$). Error bars represent standard error (SE).

our experiment and the seed morph ratio is also in our ratio range between moderate and high salinity (2.9–4.2). However, the seed morph ratio of *S. salsa* plants grown from brown seeds is *ca* 2 under 1 mmol l^{-1} NaCl (Wang *et al.*, 2015). In other words, unfavourable salinity will increase the percentage of black seeds that represent low-risk strategy. These results indicate that our salinity levels did not cause significant changes for most seed traits, and only a very low or high level of salinity could change seed morph ratio and variation of seed size in this species. Further work is necessary to determine the low and high salinity limitations for variation in seed traits.

If an experimental manipulation induces an increase in offspring size variation and retains the same mean size of their offspring, then part of the offspring should decrease to a certain minimum size threshold. Thus, mothers should increase offspring mean size (Crean and Marshall, 2009). In our study, we found support for the prediction. For each offspring seed type, seed size variability of plants from brown seeds was higher than that of plants from black seeds. Accordingly, the size of seeds produced by plants from brown seeds was significantly larger than that produced by plants from black seeds. Furthermore, when comparing offspring mean size and variation under different levels of salinity, we also find this increasing trend. The induction of an adaptive shift in offspring mean seed size is a side-effect of offspring seed size variation. This shift highlights the internal relevance of mean and variance in offspring seed size. This is the first verification of this theoretical prediction for plant traits.

In conclusion, our results suggest that the diaspore heteromorphic species *S. salsa* probably has a dual dynamic bet hedging strategy that allows it to cope with environmental uncertainty. In addition, our study is the first to show excellent agreement with the theoretical prediction that an increase in offspring size variability in seed traits increases mean size of offspring (seeds). We suggest that the dual bet hedging concept is a useful framework for understanding the life history strategies of heteromorphic species.

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Conflicts of interest. None.

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