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BRIEF COMMUNICATION

PARENTAL EFFECTS ON SEED MASS: SEED COAT BUT NOT EMBRYO/ENDOSPERM EFFECTS[1]

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Many biologists studying environmentally induced parental effects have indirectly suggested that the parental environment alters seed mass by altering the amount of endosperm or embryo tissue in the seed. We tested this hypothesis by measuring the effects of parental temperature on total seed mass, seed coat mass, and embryo/endosperm mass in offspring of *Plantago lanceolata*. Parental temperature significantly affected total seed and coat mass but not endosperm/embryo mass. Thus, larger seeds do not contain more resources in the embryo or endosperm than do small seeds. Rather they have more coat mass, which probably strongly influences germination. These results suggest caution when making assumptions about the pathways by which environmentally induced parental effects are transmitted in plant species. We also observed that controlled crosses differed significantly in their response to parental temperature, which provides evidence for genetic variation in environmentally induced parental effects, i.e., intergenerational phenotypic plasticity, in natural populations of *P. lanceolata*.

Key words: parental (maternal) effects; parental temperature; *Plantago lanceolata*; Plantaginaceae; seed mass.

Many studies of parental (maternal) effects have suggested that parental environment can alter offspring fitness by influencing the amount of resources that a maternal parent packs into its seeds. These resources represent capital that is used later by the offspring for seedling establishment and subsequent growth and reproduction (see reviews by Roach and Wulff, 1987; Gutterman, 1992; Wulff, 1995). This hypothesis is based on two observations: first, many studies of parental effects show that parental environment influences seed mass; second, many other studies show that seed mass can strongly influence growth, competitive ability, and other fitness traits (see references cited in above reviews). These observations suggest that seeds enlarged by the parental environment have more stored resources in the endosperm, or embryo, than do smaller seeds.

To test this hypothesis, we examined the tissue(s) involved in mediating the effect of parental temperature on offspring seed mass in *Plantago lanceolata* L. (Plantaginaceae), a cosmopolitan temperate herbaceous plant species. The experiment that we describe here complements a study that has already documented the effects of parental temperature on a suite of life-history traits in this species (Lacey, 1996). That study showed that parental temperature influences offspring seed mass, germination, growth, and flowering time. With respect to seed mass, low parental temperature increases seed mass. Parental environment could theoretically alter seed mass by altering the mass of any one or all three of the tissues constituting the seed: the seed coat, the embryo, or the endosperm (e.g., Roach and Wulff, 1987; Lacey, 1991, 1996; Schmid and Dolt, 1994). In the experiment reported here, we measured the effect of parental temperature on offspring coat mass and embryo/endosperm mass to determine their roles in mediating the parental effects on total seed mass. We also looked for genotypic differences in response to parental temperature as measured in terms of coat and embryo/endosperm mass.

METHODS

Details of the selection, growing conditions, crossing design, and temperature treatments of plants used for the first and second generations are given in Lacey (1996). Here we summarize features that are particularly relevant to this experiment. Five plaintain genotypes collected from one population and four from another population were cloned, and ramets of each clone were randomly assigned to one of two growth chambers that differed in their temperature regime: low = 15°C nights/20°C days, high = 20°C nights/26°C days. Both chambers were set for the same light and humidity values. Because of careful daily monitoring of temperature, light, and humidity and because plants and temperature settings were switched between chambers each month, we assumed that the temperature differences between chambers exceeded other possible chamber effects.

After ~2 mo, all plants were induced to flower and reciprocally crossed using a Comstock-Robinson type II mating design. Each genotype from one population was mated with each genotype from the other population, with each genotype being used both maternally and paternally, but in different crosses. We will use the word "CROSS" to refer to the mating of a genotype from one population with a genotype from the other population. Thus, a CROSS includes the reciprocal crosses for a pair of genotypes.

Crosses were made both within and between chambers so that the importance of maternal, paternal, and postzygotic temperatures could

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TABLE 1. Effects of temperature treatment (TRT) and cross on total seed mass, coat mass, and endosperm/embryo (EE) mass in *Plantago lanceolata*. The r^2 values for the full fixed-model ANOVAs were 0.79, 0.84, and 0.78, respectively. The contrasts show significance levels for the maternal, paternal, and postzygotic effects. For all contrasts, df = 1. Significance levels: † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = $P \geq 0.10$. We show separately the significance levels for both fixed- and mixed-models (fixed-model/mixed-model) only for those cases where the significance levels differed.

A) Full model

Source	df	Seed (MS)	Coat (MS)	EE (MS)
TRT	5	106 382*/ns	6565***	68 799
CROSS	10	58 044	1217	48 996
TRT × CROSS	50	74 626*	1394*	57 388†
Error	30	41 648	736	33 738

B) Treatment contrasts

Source	TRTs	Seed	Coat	EE
Maternal prezygotic	1 vs. 2	ns	ns	ns
	3 vs. 4	ns	ns	ns
Paternal prezygotic	1 vs. 6	ns	†	ns
	4 vs. 5	ns	ns	ns
Postzygotic	2 vs. 5	ns	**	ns
	3 vs. 6	ns	***	ns

be independently measured. The crossing design yielded six temperature treatments (TRTs), each characterized by a unique combination of maternal prezygotic, paternal prezygotic, and postzygotic temperatures (shown at the bottom of Figs. 1–2). The maternal and paternal prezygotic temperatures were the temperatures under which the mother and father, respectively, were grown before pollination, and the postzygotic temperature was the temperature during pollination and seed maturation on the maternal parent.

Twelve seeds per reciprocal cross per TRT were individually weighed to the nearest 0.1 mg and germinated, and seed coats retrieved. We obtained the embryo/endosperm mass for each seed by subtracting the coat mass from the total seed mass. Fertilized ovules develop into seeds that are composed of an embryo, surrounding endosperm tissue, and a seed coat (Cooper, 1942). Typically, a seed coat drops off a seedling soon after germination. In cases where a coat remains attached to a cotyledon, it can usually be easily removed with forceps. We collected only coats that we slipped off cotyledons easily. This ensured that we knew the parentage of the coats and also reduced the likelihood of removing cotyledon tissue along with the coat. We discarded any coat that resisted removal and any coat removed from cotyledon leaves that did not look intact after coat removal. We also discarded any coat that fell off the cotyledons between collection times. Coat mass data were obtained for all TRTs for 11 CROSSes. Thus, the analysis was performed on a subset of the crosses that Lacey (1996) used for her experiment.

We used fixed- and mixed-model analyses of variance (GLM procedures; SAS, 1985) to examine the effects of TRT and CROSS on total seed, coat, and embryo/endosperm mass. We performed each ANOVA on the mean values for each replicate cross by TRT combination. Each cell in the analysis contained 1–2 replicate mean values. To determine the source of the parental treatment effects, we examined six pairs of contrasts (GLM procedures, SAS, 1985): for maternal prezygotic temperature effects, we compared treatments 1 vs. 2 and 3 vs. 4 (see bottom of Fig. 1); for paternal prezygotic temperature effects, we compared treatments 1 vs. 6 and 4 vs. 5; for postzygotic effects, treatments 6 vs. 3 and 2 vs. 5. We did not use a Bonferroni procedure to adjust the P values for the contrasts because each contrast had an a priori reason to be of interest; also, only a small fraction of all possible contrasts were examined.

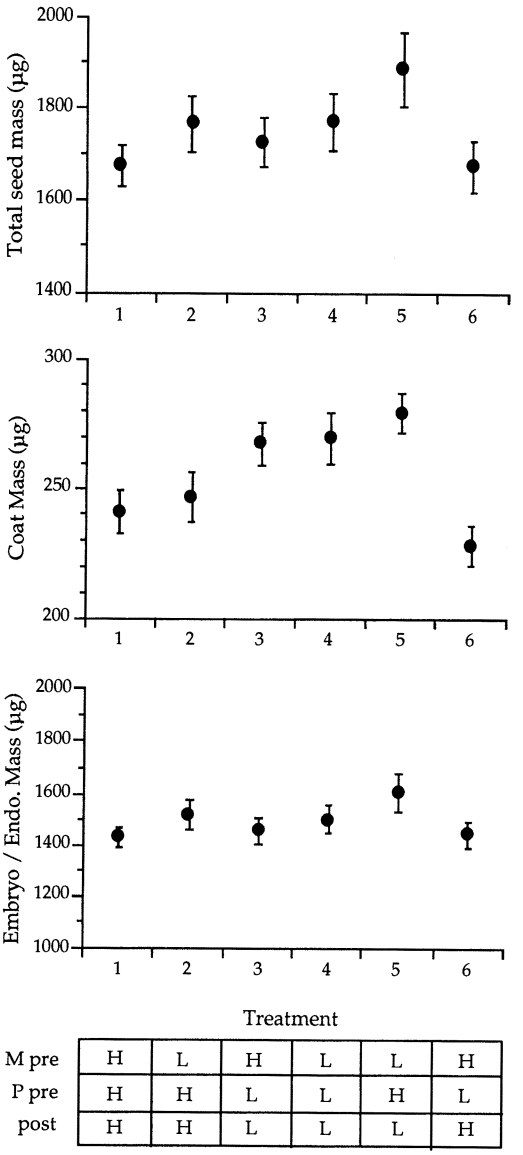
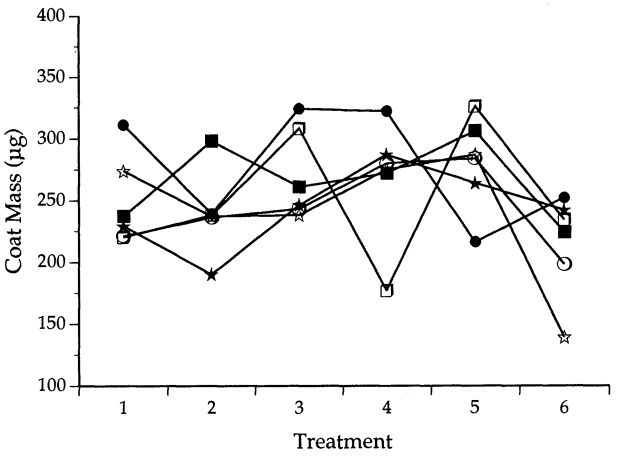


Fig. 1. Effects of first-generation temperature treatment on total seed mass, coat mass, and endosperm/embryo mass in the second generation in *P. lanceolata*. Mean values (± 1 SE) are shown for total seed, coat, and embryo/endosperm masses. Temperatures for the maternal prezygotic (M pre), paternal prezygotic (P pre), and postzygotic (post) phases of the first generation are shown for each treatment. Temperatures: L = low (20°C days/15°C nights), H = high (26°C days/20°C nights).

RESULTS

Parental temperature significantly affected total seed mass using the fixed- but not the mixed-model ANOVA (Table 1A). Low temperature increased seed mass (Fig. 1). For both fixed and mixed models, temperature significantly affected coat mass, but not embryo/endosperm mass when averaged over CROSSes (Fig. 1; Table 1A). Thus, the effect of temperature on seed mass is explained by changes in coat mass, not by changes in embryo/endosperm mass. The postzygotic temperatures best explain these significant TRT effects (Compare TRTs 2 vs. 5 and 6 vs. 3; Table 1B). High postzygotic temperature reduced



M pre	H	L	H	L	L	H
P pre	H	H	L	L	H	L
post	H	H	L	L	L	H

Fig. 2. Norms of reaction of coat mass for six representative crosses of *P. lanceolata.* Temperatures for the maternal prezygotic (M pre), paternal prezygotic (P pre), and postzygotic (post) phases of the first generation are shown for each treatment. Temperatures: L = low, H = high.

coat mass and total seed mass. Also, the reaction norms to parental temperature treatment differed significantly among CROSSes for both coat mass and total seed mass (Fig. 2, Table 1A). They differed marginally for embryo/endosperm mass ($P = 0.061$).

DISCUSSION

This experiment clearly shows that one cannot assume that larger seeds have more storage tissue (i.e., more embryo or endosperm tissue) than do smaller seeds or that a parental effect on seed mass is mediated by amount of storage tissue. In *P. lanceolata,* low parental temperature increases seed mass by increasing coat mass rather than by increasing embryo/endosperm mass. Sultan (1996) has recently observed a similar effect of increasing parental light levels on the same components of seed mass in *Polygonum persicaria.* Thus, we suggest caution when making inferences about the pathways by which environmentally induced parental effects are transmitted in plant species.

Of interest to ecologists and evolutionary biologists is not just how the parental environment affects seed tissues, per se, but also how the parent transmits environmental effects via the seed to traits more directly affecting population dynamics and individual fitness. Several lines of evidence suggest that the seed coat is mediating the parental temperature effect on germination in *P. lanceolata.* Lacey (1996) found that low parental temperature strongly reduces germination in *P. lanceolata,* and here we have shown that low temperature increases coat mass, which could be the cause of this reduction. Seed coat thickness and chemical composition and the thickness of maternal tissue surrounding the seed have been shown to regulate germination in other plant species (e.g., Evenari, 1956; Koller, 1972; Cresswell and Grime, 1981; Dorne, 1981; Gutterman, 1982, 1992). In some species both the coat and embryo control dormancy (e.g., Morley, 1958; Garbutt and Witcombe, 1986). Therefore, the regulation of germination by coat mass in *P. lanceolata* would not be surprising. Also, one would predict that any temperature effects transmitted via the seed coat should not persist past one generation, given that the coat is derived entirely from maternal tissue and is cast away from the offspring upon germination. The observation that the temperature effect on germination does not persist to the third generation when the effects on other life-history traits, e.g., flowering time, do persist (Case, Lacey, and Hopkins,1996) is consistent with this prediction.

There is abundant evidence that environmentally induced parental effects can influence the phenotypic expression of physiological, morphological, and life-history traits in plants (e.g., Roach and Wulff, 1987; Gutterman, 1992; Wulff, 1995; Case, Lacey, and Hopkins, 1996; Lacey, 1996). The mechanisms by which these effects are mediated, however, are not understood (Roach and Wulff, 1987; Gutterman, 1992; Wulff, 1995). For example, many studies suggest that the environment influences offspring phenotype during the time of fertilization and offspring seed development, i.e., during early embryonic development of an offspring while attached to its maternal parent. During this time one or several pathways of transmission could be involved (e. g., Koller, 1972; Cresswell and Grime, 1981; Gutterman, 1982; Garbutt and Witcombe, 1986; Roach and Wulff, 1987; Lacey, 1991, 1996; Schmid and Dolt, 1994). Theoretically, the environment could produce nongenetic, maternal changes in the seed coat tissue or endosperm/embryo cytoplasm or in maternal tissue that surrounds and is dispersed with the seed. Alternatively, the environment could alter gene structure or activity in either the endosperm or embryo. Changes could involve seed quantity, which would affect seed or propagule mass, and/or quality, which may or may not affect mass. It is also conceivable that an environmental factor could transmit a parental effect via one or multiple tissues depending on the traits being examined. For example, the data from this study and Case, Lacey, and Hopkins (1996) suggest that the temperature effect on germination is mediated by the seed coat but that the temperature effects on other life-history traits, which persist to the third generation, are mediated by the embryo. In general, additional research examining the mass of the seed tissues could help to discriminate among these alternative pathways of transmission.

Such research will also help us to determine whether or not the environmentally induced effects that have been reported in the literature are truly environmentally induced parental effects sensu stricto, i.e.,whether or not they represent intergenerational phenotypic plasticity (Lacey, in press). For example, Lacey (1996) observed that parental temperature affected offspring seed mass. However, she noted that this response could have been explained by an intergenerational parental effect, gametophytic/gametic selection, or an intragenerational environmental effect on offspring embryogenesis. These confounding processes could explain reported parental environmental effects on seed mass in many plant species (e.g., Mazer and Gorchov, 1996; Lacey, in press). Our

experiment helps to discriminate among these alternatives. Because the coat tissue is entirely maternally derived, the seed-mass response truly reflects an intergenerational parental effect, and consequently, intergenerational phenotypic plasticity.

The fitness consequences of this plasticity are presently unknown. However, the observed change in rank orders of families across temperature treatments for coat mass shows that the potential for evolutionary change exists in natural populations. For example, two of the three CROSSes having the lightest coat masses at high temperature (TRT 1 in Fig. 2) had among the heaviest coat masses at low temperature (TRT 4). If coat mass influences offspring fitness via its effect on germination, then environments having different temperature regimes should select for different families based on coat mass. Whether or not this actually happens remains to be determined.

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