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The genetics and ecology of seed size variation in a biennial plant, *Hydrophyllum appendiculatum* (Hydrophyllaceae)

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Abstract The goal of this study was to elucidate the sources of seed size variation in *Hydrophyllum appendiculatum*, an outcrossing, biennial plant. The genetic basis of seed size variation was examined with a diallel breeding design. The analysis did not reveal any evidence for additive genetic variance, suggesting that seed size could not evolve in response to natural selection. A series of greenhouse experiments was conducted to determine the sensitivity of seed weight to a number of ecological variables. Seed weight was affected by inbreeding depression: seeds produced by self-pollinations were significantly lighter than outcrossed seeds. Maternal plants did not differentially provision seeds that were the result of crosses between subpopulations (separated by 300 m) or between populations (separated by 1.7 km). Mean seed size was independent of the number of outcrossed pollen donors (one vs. many) that sired seeds on an inflorescence; however, the variance was greater on inflorescences pollinated by multiple donors. Direct manipulations of the abiotic environment showed that seed size was greater on plants growing under full sunlight compared to shaded plants. Seed size was unaffected by soil type, fertilizer addition, or defoliation. Finally, I determined the effect of varying pollination intensity at the level of a single inflorescence, and at the whole plant level. Seed weight was greatest on plants that had only 1 and 5 inflorescences pollinated, and least on those that had 10 and 20 pollinated. At the inflorescence level, seed weights were greatest on those where all flowers were pollinated, compared to inflorescences where only half of the flowers were pollinated. Perhaps the greatest contributor to variance in seed size in this species was the temporal decline within plants through the flowering season. These results indicate that maternal plants are not capable of producing uniform seed crops. Rather, the final distribution of seed size produced by plants within a population will necessarily vary and be the result of pollination effects, heterogeneity in the abiotic environment, and developmental constraints.

Key words *Hydrophyllum appendiculatum* · Seed size Flower size · Maternal effects · Pollen donor effects

Introduction

Adult fitness hierarchies are the result of a series of processes and events that occur between birth and reproduction. In angiosperms, intial seed weight has a strong effect on plant success. Typically, large seeds produce large seedlings (Harper and Obeid 1967; Weis 1982; Gross 1984; Schemske 1984; Waller 1985; Gross and Kromer 1986; Wulff 1986; Dawson and Ehleringer 1991) and this larger juvenile size enhances survival probability, adult size, and fecundity (Howe and Richter 1982; Pitelka et al. 1983; Dolan 1984; Glewen and Vogel 1984; Stanton 1984, 1985; Roach 1986; Mazer 1987). Given the importance of seed weight to eventual plant fitness, it is important to understand the forces responsible for the high degree of variation within species observed in this trait.

One of the critical issues concerning phenotypic variation in any trait lies in determining whether there is a genetic basis to the variation. The few studies that have investigated heritability of seed size in wild species typically report little or no variance among paternal families (half-sibships) or additive genetic variance (Schaal 1980; Antonovics and Schmitt 1986; Mazer 1987; Schwaegerle and Levin 1990; Mazer and Wolfe

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1992). In contrast, maternal plant effects on seed weight are usually great (Antonovics and Schmitt 1986; Marshall and Ellstrand 1986; Mazer et al. 1986; Weis et al. 1987; Nakamura and Stanton 1989; Stratton 1989; Mazer and Wolfe 1992) and probably due to the large contribution of genetic (half of the genes to the embryo, two-thirds to the endosperm) and non-genetic (cytoplasm and seed coat) materials by the mother. Furthermore, maternal plants also provide the environment in which seeds spend their entire development period (Gutterman 1980–1981; Roach and Wulff 1987; Devlin 1988). As such, maturing seeds are susceptible to features of the environment experienced by the maternal plant, including temperature (Alexander and Wulff 1985; Wulff 1986), nutrient levels (Parrish and Bazzaz 1985; Stratton 1989), photoperiod (Cook 1975) and herbivory (Maun and Cavers 1971; Bentley et al. 1980; but see Thompson and Pellmyr 1989).

The quality of the maternal environment, or the provisioning ability of the maternal plant, can also be affected by the level of competition among developing seeds. Pollination intensity experiments have shown that seed weight increases as the number of flowers pollinated decreases (Pederson et al. 1956; Stanton et al. 1987; Lalonde and Roitberg 1989). Variable-sized progenies could also arise if maternal plants regulate investment to individual offspring based on some measure of their genetic makeup (e.g., product of selfed vs. outcrossed mating: Brink and Cooper 1940; Sorenson and Miles 1974; Schemske and Pautler 1984; Galen et al. 1985; Kalisz 1989; but see Winn 1991), genetic quality (Westoby and Rice 1982) or variability among pollen donors (Price and Waser 1979; Janzen et al. 1980; Levin 1984; Schemske and Pautler 1984; Marshall and Ellstrand 1986; Temme 1986).

While it is clear that a number of genetic and environmental factors influence seed weight, and that this trait has important implications for the ecology of plant populations, there are few examples of species for which we know the ecological consequences, genetic control and ecological causes of phenotypic variation in seed size. In this paper I examine several aspects of seed size variation in *Hydrophyllum appendiculatum* (Hydrophyllaceae), a biennial species in which seed size varies ten-fold and is positively correlated with juvenile size and survival (Wolfe 1990, 1993a). Specifically, I addressed the following questions:

1. Does additive genetic variation exist for seed weight? By using a half-sib analysis (diallel breeding design) I determined if seed size is heritable and could potentially respond to natural selection.
2. Does pollen source affect seed size? Here I was interested in the influence of three genetic effects associated with the identity of pollen: inbreeding depression (self vs. outcross pollination); physical distance between donors and recipients (intra- vs. interpopulation crosses); and single vs. multiple outcross donors.
3. Is seed size sensitive to attributes of the micro-environment of the maternal plant (e.g., light levels, soil quality)?
4. Does varying pollination intensity, at either the level of the inflorescence or entire plant, influence seed size?
5. Is seed size constant through the flowering season within an individual plant and does it vary with flower size?

Materials and methods

The study organism

Hydrophyllum appendiculatum is an understory, spring-flowering species that grows in deciduous woods of the midwest United States. Flowering occurs while the forest canopy is leafing out and seed maturation is completed under a fully-closed canopy. Rosettes form soon after seedling emergence in spring, overwinter, and bolt in their second spring. *H. appendiculatum* behaves as an obligate biennial since all surviving individuals reproduce, regardless of size (Wolfe 1993a). The blue flowers contain a single ovule: as a result, fruit set is equivalent to seed set. Individual flowers remain open for 2–3 days and are pollinated primarily by honey bees (Wolfe 1993b). Under field conditions plants produce 1 to about 25 inflorescences, each composed of about 20 flowers. *H. appendiculatum* is protandrous, completely self-compatible, and primarily outcrossing (Wolfe and Shore 1992).

Plant material used in this study was collected from Trelease Woods and Brownfield Woods which are located approximately 10 km north-east Urbana, Illinois (Champaign County). These two forests are separated by 1.7 km of corn and soybean fields. Both forests are maintained by the University of Illinois as research areas.

General protocol

The pollination experiments described below were conducted in the greenhouse and several used similar methods. Pre-reproductive, 2nd-year rosettes were typically removed from either Trelease Woods or Brownfield Woods soon after snowmelt in March and prior to bolting. Except where otherwise indicated, transplants were placed into 18-cm pots containing a standard greenhouse soil mix (1 peat:2 loam:1 perlite). Flowering usually began about 3 weeks after transplanting. Pollinations were conducted by removing stamens with forceps and pressing fully dehisced anthers onto stigmas. Following completion of the pollination program, inflorescences were enclosed in bridal veil bags for seed collection. All plants were moved regularly during the pollination program and period of seed maturation so that greenhouse positional effects on seed size would be minimized. Following maturation, seeds were individually weighed to the nearest mg.

All analyses in this study were conducted with SAS (SAS Institute 1985). Transformations were not required since seed weight was always normally distributed. Analyses of variance (ANOVA) were performed with the GLM procedure and type III sums of squares. The specifics of each ANOVA are described in the appropriate section below. Multiple comparisons were performed with Tukey's test.

Genetic control of seed weight – Half-sib analysis

A diallel breeding design was used to create maternal and paternal half-sib progenies to partition the phenotypic variation of seed weight into components attributable to the maternal parent, paternal parent, and the interaction between them. Assuming that cytoplasmic inheritance from pollen donors is negligible, any differences among paternal half-sibships would be due to additive genetic effects. In contrast, since a large portion of the angiosperm seed is derived directly from the maternal parent, differences among maternal half-sibships could be due to additive effects as well as maternal environmental and maternal genetic effects (Dickerson 1969).

In early March 1990, nine prereproductive plants were transplanted from Trelease Woods. Each inflorescence used on these plants received pollen from the eight other plants. Individual flowers received pollen from just one donor and were individually tagged with colored thread to denote the donor. Approximately 1500 flowers were pollinated.

Maternal and paternal plant effects were considered random and tested over the interaction term in the two-way ANOVA. Variance component estimates were generated using the Random statement in the GLM procedure of SAS.

Pollen donor effects

Selfing versus single and multiple outcrossed pollen donors

The purpose of this experiment was to determine if the identity (self vs. outcross) and number of outcrossed pollen donors (single vs. multiply-sired inflorescences) influences mean seed weight in *H. appendiculatum*. This experiment also addressed whether increasing the diversity of pollen donors results in increased variability in offspring weight. In early March 1987, six prereproductive individuals were transplanted into the greenhouse. Inflorescences on each plant were subjected to one of three treatments: (1) all flowers on an inflorescence self-pollinated; (2) all flowers on an inflorescence pollinated with pollen from one other individual (single outcross pollen donor); (3) each flower on an inflorescence pollinated with pollen taken from a different pollen donor (multiple outcross pollen donors). Other plants removed from the same area in Trelease Woods were used as additional pollen sources. Each treatment was replicated three times per plant to yield a total of 706 seeds. Data were analyzed using a two-way mixed ANOVA with maternal plant considered as a random effect and pollination treatment as a fixed effect. Here, and throughout this paper, in two-way mixed ANOVAs, the fixed effect was tested over the interaction term (Sokal and Rohlf 1981, p. 340). The degree of variability in seed weight produced by single and multiple-sired inflorescences was examined with the coefficient of variation (CV).

Spatial effects: intra- and interpopulation crosses

This experiment was conducted to determine if the physical distance separating pollen donors and maternal parents influences offspring size. This greenhouse study was performed by conducting pollinations between plants taken from: 1. the same subpopulation in Trelease Woods; 2. between subpopulations in Trelease Woods; and 3. between plants from Trelease and Brownfield Woods. In the spring of 1987, I collected 13 pre-reproductive plants from each of two areas in Trelease Woods separated by approximately 300 m (Trelease North and Trelease East), and 10 plants from Brownfield Woods. To reduce the number of possible pollinations, only the plants from the two subpopulations in Trelease served as pollen-receiving or maternal parents; Brownfield plants served only as pollen donors. All maternal plants received pollen from multiple individuals within each of the three donor groups. This pollination program created 518 seeds. Data were analyzed with a two-way ANOVA, with site of pollen source and seed source considered fixed effects.

Maternal environmental sources of variation

Variable maternal environment

Three abiotic characteristics of the maternal plants were manipulated in a completely crossed design (eight treatment groups): 1. light level [normal greenhouse levels vs. shaded (50% shade cloth)]; 2. soil type [high nutrient content (standard greenhouse mix) vs. low nutrient content (3 calcite clay: 1 standard greenhouse mix)]; 3. fertilizer [not fertilized vs. fertilized (weekly applications of 15 ml/l 20:20:20 NPK in solution)].

In early March 1988, 80 rosettes were transplanted and randomly assigned to one of the eight treatment groups. Weekly additions of fertilizer began 1 week after transplanting. All plants were maintained under experimental conditions through flowering and seed maturation.

As the plants came into flower the first two inflorescences of each plant were self-pollinated to produce seeds on maternal plants raised under different conditions. Approximately 2000 pollinations were conducted. For each maternal plant I recorded plant size (number of leaves) at various times during the life cycle, time of first flower, length of the longest leaf at the onset of flowering, number of inflorescences produced, flower size (petal length, one per inflorescence), seed set, and mean seed weight. Data were analyzed using a three-way ANOVA with light level, soil type, and fertilizer treatment considered fixed effects. Percent seed set was arcsine-transformed prior to analysis.

Defoliation

This experiment was conducted to determine if removal of leaves subtending seed-bearing infructescences would affect seed weight. In early March 1988, 33 rosettes were randomly assorted into three treatment groups: 1. control (no leaves removed); 2. the closest leaf to the inflorescence removed; 3. the five closest leaves removed. Leaves were removed with scissors just prior to flowering. At the time of defoliation, all plants were of similar size and had at least 25 leaves. Only one inflorescence was self-pollinated on each plant. Data were analyzed using a nested ANOVA, with maternal plant nested under the defoliation treatment.

Pollination intensity: at the inflorescence level

If seed weight is controlled at the level of the individual inflorescence, then it may decline as the number of flowers pollinated on each inflorescence increases. In early March 1988, 13 rosettes of *H. appendiculatum* were transplanted into the greenhouse. All plants received two replicates (= inflorescence) of each of two self pollination treatments: 1. half of the flowers on an inflorescence pollinated; 2. all flowers on an inflorescence pollinated.

Pollination intensity: at the plant level

If resources available to a maternal plant are finite, then seed weight may decline as the number of seeds competing for those resources increases. In March 1989, 28 rosettes were randomly assorted to one of four pollination treatments: either 1, 5, 10, or 20 inflorescences were pollinated per plant. All flowers on the appropriate number of inflorescences were pollinated with self pollen.

Seasonal pattern of seed weight within plants

A pollination program was initiated in 1986 to produce progenies from mothers to determine if seed weight varies through the flowering season. In March 1986, 22 prereproductive plants were transplanted from Trelease Woods into the greenhouse. Flowers on these plants were pollinated with pollen taken randomly from the other 21 donor plants. Pollinations were performed daily for about 2 months. At least 15 inflorescences on each plant were pollinated. Each inflorescence was tagged with a colored thread and the date of first flower opening for each inflorescence was recorded. Since each inflorescence was tagged uniquely, I was able to determine when seeds were produced during the season.

Results

Genetic control

The results of the diallel breeding design showed that a significant portion of the variation in individual seed weight was explained by differences among maternal families (Table 1). In contrast, no significant differences were detected among paternal families. Variance-component analysis showed that the identity of the maternal plant explained 33% of the variance in seed weight and paternal parent explained less than 1% of the variance. The lack of variation in seed weight among paternal half-sib families indicates that there is little or no additive genetic variance for seed weight in this species.

Table 1 ANOVA results of a diallel breeding design examining the contribution of maternal and paternal parents to seed weight variation in *Hydrophyllum appendiculatum*

Source	*df*	MS	*F*	*P*	%Variance explained
Maternal parent	8	1666.55	12.63	0.0001	33.1
Paternal parent	8	165.12	1.25	0.25	0.9
Maternal × Paternal	52	131.91	3.54	0.288	13.5
Error	638	37.31			52.4

Table 2 Effect of different pollen donor types on seed weight (mg) in *H. appendiculatum*. The pollen type effect in the ANOVA represents the three pollen treatments applied to whole inflorescences: self, single outcross donor, multiple outcross donor. Means followed by the same letter are not significantly different ($P < 0.05$; Tukey's test)

A. Descriptive statistics

Pollen donor	Mean ± SD
Self	26.6 ± 7.7^{a}
Single outcross	30.3 ± 7.7^{b}
Multiple outcross	29.5 ± 8.1^{b}

B. ANOVA

Source	*df*	MS	*F*	*P*
Maternal plant	5	19999.99	44.36	0.0001
Pollen type	2	1083.21	24.02	0.0001
Plant × pollen	10	126.19	2.80	0.0021
Error	688	45.08		

Table 3 The effect of intra- and interpopulation crosses on seed weight in *H. appendiculatum*. Plants from the two Trelease Woods (East, North) sites served as seed parents; plants from Brownfield Woods were used only as pollen sources

A. Descriptive statistics

Seed parent	Pollen source	Mean ± SD
Trelease East	Trelease East	36.2 ± 8.13
Trelease East	Trelease North	34.3 ± 8.44
Trelease East	Brownfield	34.2 ± 8.80
Trelease North	Trelease North	36.9 ± 6.61
Trelease North	Trelease East	36.1 ± 6.07
Trelease North	Brownfield	36.6 ± 7.85

B. ANOVA

Source	*df*	MS	*F*	*P*
Maternal origin	1	373.30	6.28	0.012
Pollen origin	2	26.88	0.45	0.636
Seed × pollen	2	95.23	1.60	0.202
Error	513	59.46		

Pollen donor effects

Breeding system had a significant effect on seed weight in *H. appendiculatum* (Table 2). Outcrossed seeds (pooled single and multiple outcross) were heavier than selfed seeds (29.9 ± 7.7 vs. 26.6 ± 7.7 mg, $t = 5.36$, $P < 0.001$). Seeds produced on inflorescences in which every flower received pollen from a different donor were not significantly different in weight from those that were pollinated by just a single donor (Table 2). The ANOVA revealed a significant interaction between individual maternal plant and pollination type ($P < 0.0021$; Table 2). The magnitude of variability in seed weight was greater for multiply-sired infructescences than for singly-sired infructescences at the probability level of 0.10 (CV = 21.93 ±9.59, $n = 27$ vs. 17.99 ± 8.45, $n = 29$, respectively; $t = 1.62$). The CV was greatest for self-ed seeds (23.81±12.48, $n = 42$).

There were no significant effects of spatial separation between parents on seed weight. Overall, seed weight was independent of the site from which pollen originated (Table 3). There was also no difference in the level of seed set arising from pollinations within or between different sites.

Maternal parent effects on seed weight – manipulations of maternal plants

Growing plants under different micro-environmental conditions affected vegetative and reproductive traits. Overall, light level had the most pronounced and consistent effects compared to soil type and fertilizer addition. Plants growing under full light conditions were larger at flowering, flowered earlier, produced more inflorescences, had larger flowers and higher levels of seed set than those growing under shady conditions (Tables 4 and 5). Despite large effects of fertilization,

Table 4 The effect of various microenvironmental conditions on vegetative and reproductive characters in *H. appendiculatum*. Values are means ± SD. Pairs of means that are underlined are significantly different ($P < 0.05$, Tukey's test)

Trait	Light level Shaded	Full sun	Soil type Low nutrient	High nutrient	Fertilizer added No	Yes
Leaves at start of exp.	3.8 ± 1.3	4.1 ± 1.5	4.0 ± 1.4	3.8 ± 1.4	4.2 ± 1.4	3.6 ± 1.3
Leaves at first flower	4.5 ± 2.4	35.9 ± 25.8	26.8 ± 23.8	32.3 ± 28.9	12.8 ± 8.4	50.0 ± 26.4
Length long leaf (cm)	7.8 ± 2.1	9.3 ± 1.8	8.5 ± 2.1	9.3 ± 1.9	8.5 ± 1.8	9.2 ± 2.1
Day of first flower	26.5 ± 4.1	16.3 ± 3.0	20.4 ± 6.0	19.9 ± 6.2	19.5 ± 6.1	20.7 ± 6.0
Inflorescences (#)	5.5 ± 3.6	32.6 ± 26.7	27.5 ± 27	27.6 ± 26.1	9.7 ± 6.0	48.6 ± 25.4
Flower size (mm)	9.1 ± 1.9	12.8 ± 1.0	10.2 ± 2.3	11.0 ± 2.6	10.8 ± 1.9	10.2 ± 3.1
Seed set (%)	41.0 ± 21	56.0 ± 13	48.0 ± 17	57.0 ± 15	52.9 ± 12.3	52.6 ± 19.6
Seed weight (mg)	24.9 ± 4.2	33.0 ± 6.9	29.9 ± 6.7	32.1 ± 7.3	29.0 ± 6.0	33.0 ± 7.0

Table 5 The effect of different microenvironments on plant size and reproductive traits in *H. appendiculatum*. Numbers are *F* values ample size (*n* plants) is given in parentheses

Source	Size at start (80)	Size at flower (50)	Day first flower (50)	Flower size (50)	Number of inflorescences (50)	Seed set (50)	Seed weight (50)
Light	0.92	29.31***	90.68***	103.09***	14.53**	8.70**	16.47***
Soil	0.64	4.13*	0.20	0.55	0.12	10.79**	0.75
Fertilizer	2.66	5.88**	0.94	5.75*	9.87**	0.05	0.12
Light × soil	0.01	3.55	1.50	2.36	0.27	4.64**	0.01
Light × fert	1.68	5.47*	0.03	10.15**	8.18**	0.09	2.35
Soil × fert	0.41	3.90*	2.00	0.22	0.75	8.81**	0.64
Light × soil × fert	0.23	0.92	0.02	12.29**	0.05	1.83	0.52

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$

Table 6 Effect of removal of different numbers of subtending leaves (defoliation level) on seed weight (mg) in *H. appendiculatum*. The control group had no leaves removed

A. Descriptive statistics

Defoliation level	Mean ± SD
None	33.5 ± 8.3
One	36.9 ± 7.6
Five	34.7 ± 6.9

B. ANOVA

Source	*df*	MS	*F*	*P*
Defoliation	2	155.27	0.345	> 0.5
Plant (defol)	31	449.29	7.48	0.0001
Error	631	60.09		

Table 7 Pollination intensity within inflorescences. The effect of pollinating half or all of the flowers on an inflorescence on seed weight (mg) in *H. appendiculatum*

A. Descriptive statistics

	Fraction of flowers pollinated Half	All	*t*	*P*
Seed weight	26.5 ± 7.8	29.2 ± 8.1	2.78	0.01

B. ANOVA

Source	*df*	MS	*F*	*P*
Plant	12	682.9	19.73	0.0001
Pollination intensity	1	454.48	10.75	0.01
Plant × poll. int.	11	42.27	1.22	0.25
Error	304	34.61		

seed weight was significantly influenced only by light level: plants in full sun producing heavier seeds than shaded plants.

Using only those plants growing under full light, there was a significant relationship between flower size and the mean weight of seeds produced (Fig. 1).

Defoliation did not affect seed weight (Table 6). Seed weight was not significantly different among plants that had none, one, or five of the nearest leaves removed from developing infructescences ($P > 0.5$, Table 6).

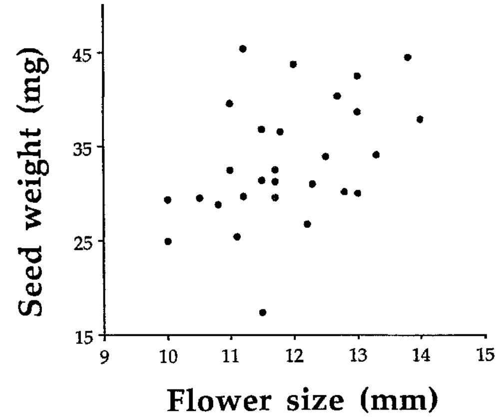

Fig. 1 The relationship between flower size and subsequent seed weight in *Hydrophyllum appendiculatum*. Each value is a family mean, $r = 0.431$, $P \leq 0.022$

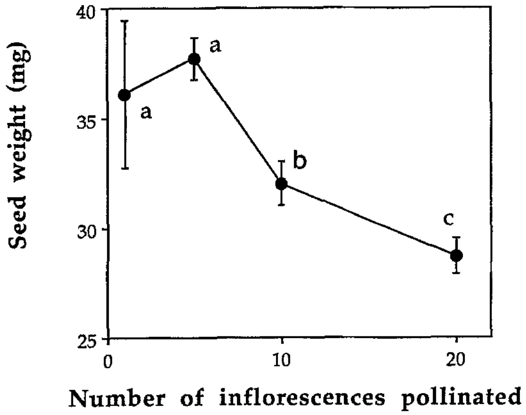

Fig. 2 The relationship between pollination intensity (number of inflorescences pollinated) and seed weight (mean ± 2SE) in *Hydrophyllum appendiculatum*. Means with the same *letter* are not significantly different (Tukey's test)

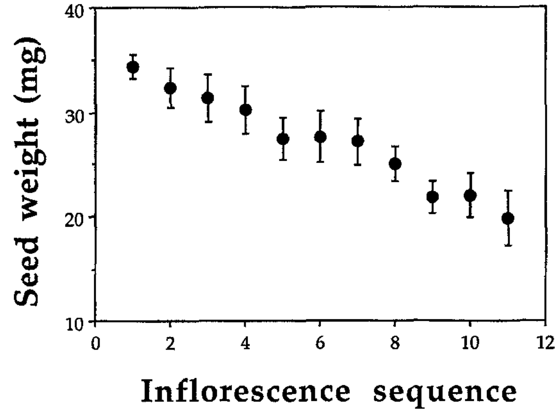

Fig. 3 The pattern of seasonal decline of seed weight in *Hydrophyllum appendiculatum*. Values are mean ± SE, $r = -0.562$, $P \leq 0.001$

Pollination intensity

At the inflorescence level

Varying the fraction of flowers pollinated on an inflorescence affected seed weight (Table 7). Seed weights were significantly heavier on those inflorescences on which all flowers were pollinated compared to those in which only half of the flowers were pollinated.

At the plant level

Seed weight was influenced by the number of inflorescences pollinated per plant. The mean seed weight on plants with 10 and 20 inflorescences pollinated was significantly less than on plants in which only one or five inflorescences produced seed ($F = 7.17$, $P < 0.001$; Fig. 2). Plants that had more inflorescences pollinated produced more seeds and there was a significant negative correlation between mean seed weight and total seed number (Pearson's $r = -0.584$, $P < 0.01$).

Seasonal pattern in seed weight

There was a strong seasonal pattern in seed weight within individual plants. Seed weight declined continuously through the flowering season on successively produced inflorescences (Pearson's $r = -0.562$, $P < 0.001$; Fig. 3).

Discussion

Seed size variation is one factor responsible for the creation of early size hierarchies in natural populations of *Hydrophyllum appendiculatum* (Wolfe 1990). To a large extent, distributions of seedling size translate into distributions of reproductive success in adults. Yet, despite the clear selective advantage accrued to large seeds, the lack of significant differences among paternal half-sib families suggests that little or no detectable additive genetic variance exists and that seed size in *H. appendiculatum* will apparently be unable to respond to natural selection. Of course it is possible that seed size is heritable and that the plants used in the diallel design were by chance not representative of the population at large. However, this same experimental design was replicated on another group of plants with identical results (Wolfe 1990). Although the pollen donor experiments showed that there is some genetic basis to the observed variation, it seems that for this species seed size variation is mainly ecological and environmental in origin. This variation stems from factors that act to increase variance both among and within plants.

Maternal environmental effects

Apart from their genetic and nutritional contribution, maternal plants also provide an environment for growing embryos. As such, the seed is not merely a *product* of the maternal plant, it may also be considered a *part* of the maternal plant. It is therefore not surprising that seed weight is so susceptible to maternal effects that overwhelm the relatively small nuclear contribution of paternal parents. This seems to be true for a wide range of angiosperms where variation among maternal plants explains more variation in seed weight than variation among paternal plants (Antonovics and Schmitt 1986; Marshall and Ellstrand 1986; Mazer 1987; Nieuwhof et al. 1989; Pittman and Levin 1989; Mazer and Wolfe 1992). In the present study, significant variation existed in mean progeny weight among maternal plants within each experiment (ANOVA, $P < 0.001$, in all cases. Significant variation also occurs among plants growing under field conditions (Wolfe 1990). Thus even when growing under conditions as uniform as possible, there are underlying differences among maternal plants. These may arise from non-additive genetically based differences in the ability to provision developing seeds.

One source of maternal effects is the physical or abiotic environment. Variation in light level was the only physical factor that influenced seed weight in *H. appendiculatum*: plants growing in full sun produced heavier seeds than did shaded plants. Light level is one environmental factor that varies extensively in the forest understory, the natural habitat of *H. appendiculatum*. This is especially true for Trelease and Brownfield Woods where the high incidence of treefalls creates a very heterogeneous light environment. Such heterogeneity will serve to increase variance among individual plants within a natural population. Furthermore, light levels change considerably during the period of flowering and seed maturation in the field as the forest canopy develops from being completely open to completely closed. Pitelka et al. (1980) have also attributed seed size variation in *Aster acuminatus* to temporal and spatial variation in light levels. Presumably, light levels affect seed weight by influencing photosynthetic rates and the amount of provisioning materials available to maternal plants. Thus, it is surprising that leaf removal in the defoliation experiment did not affect seed weight since subtending leaves tend to contribute most of the carbohydrates to developing seeds in some species (Pate and Farrington 1981). Defoliation can be high in Trelease and Brownfield Woods due to herbivory by deer (personal observation). It is possible that partial leaf removal in this species results in an increase in the photosynthetic rate in remaining leaves (Wareing et al. 1968). The absence of a defoliation effect, combined with a significant light intensity effect, suggests that developing fruits contribute photosynthetically to their seeds. Other studies have reported conflicting results with defoliation experiments: leaf removal resulted in decreased seed size in *Rumex obtusifolius* (Maun and Cavers 1971) but actually increased seed size in *Desmodium paniculatum* (Wulff 1986). In contrast to the role of light, seed weight in *H. appendiculatum* was unaffected by soil nutrient quality. This is surprising since traits such as plant size, inflorescence production and seed set were affected by either fertilizer or soil type. Clearly, various plant traits are differentially affected by characteristics of the maternal plant's abiotic environment. This indicates that either seed weight is buffered against variation in soil nutrient quality, or that there were sufficient resources available in the unfertilized and calcite-clay soil such that plants were not nutrient-limited.

Influence of pollination biology

This study has demonstrated that several features of the pollination biology of *H. appendiculatum* influence seed weight patterns. First, seeds produced from outcross pollinations were heavier than those from self pollinations. Wolfe and Shore (1992) found that although this species is primarily outcrossing, significant selfing does occur in Trelease and Brownfield populations of *H. appendiculatum*. Variation in inbreeding history among maternal plants may explain the significant interaction term between plant and pollination type seen in Table 2. It is important to note that the selfing rate in these populations varies among individual plants due to differences in the size of the floral display and also through the flowering phenology (Wolfe and Shore 1992). Consequently, the degree of inbreeding depression expressed by seed size will potentially vary both spatially and temporally.

It is difficult to determine which component of the seed is affected by the congruity of the male and female gametophytes. Brink and Cooper (1940) discovered that the greater size of outcrossed seeds in *Medicago sativa* was not due to any differences between the two embryo types, but rather to the increased growth of outcrossed endosperm. This suggests that the endosperm can serve to amplify variation among offspring. The same process may occur in *H. appendiculatum* since the embryo is barely visible in seed cross-sections and the endosperm constitutes the bulk of the seed biomass (personal observation). Second, pollinator foraging behavior can influence the diversity of pollen donors that contribute to a maternal family. As a result of visitation and grooming behavior, there will be mixed loads of pollen from different donor plants on pollinator bodies (Wolfe and Barrett 1989; Shore 1993). Depending on the number of flowers visited per plant and on the frequency of grooming, the composition of pollen deposited on stigmas can consist of pollen from one to many different outcrossed donors. By sampling gametophytes from a wider range of fathers, maternal plants may produce more variable progeny (Kress 1981; Temme 1986). This was the case for *H. appendiculatum*: the variation in seed weight produced by multiply-sired inflorescences was slightly greater than for single-sired inflorescences. However, mean seed weight was unaffected by the number of outcrossed pollen donors applied to inflorescences. An identical result was reported for *Campsis radicans* where the variance in seed weight, but not the mean, was greater in fruits sired by three pollen donors compared to single pollen donors (Bertin 1986).

Another aspect of the pollination biology that may directly affect seed size is the physical distance between pollen donors and recipients. Although most matings in a natural plant population tend to be among near neighbors (e.g., Schaal 1978), occasional matings may occur among distantly related individuals. As a result of restricted pollen and seed dispersal (Levin and Kerster 1974), plant populations tend to develop a local structure which results in relatedness between any two individuals declining with distance (Turner et al. 1982). Furthermore, localized selection may act to increase the degree of population subdivision (Antonovics

1971). The outcome of a cross between two individuals can then be seen as a compromise between inbreeding depression and outbreeding depression, with seed production and fitness greatest at intermediate distances. Despite the importance of distance-related effects in some species (Price and Waser 1979; Levin 1984; Schemske and Pautler 1984; Sobrevila 1988; but see Lee and Bazzaz 1982; Newport 1989) no differences were found in seed size (or seed set, Wolfe 1990) following outcrossing between subpopulations within Trelease, or between Trelease and Brownfield Woods. The lack of any distance-dependent effects suggests that either: (1) the degree of relationship between individuals is not accurately associated with distance; (2) the sampled populations are not subdivided; or (3) the differences among subpopulations or populations are not sufficient to allow maternal plants to distinguish among different outcrossed donors.

In addition to the role pollination plays in determining genetic consequences, the intensity of pollination will directly affect the number of flowers pollinated. This, in turn, will influence the ability of the maternal plant to provision developing seeds. Experiments in which the number of flowers pollinated have been manipulated usually reveal significant negative correlations between seed number and seed weight (e.g., Pederson et al. 1956; Lalonde and Roitberg 1989). The same relationship exists in *H. appendiculatum* as plants with more inflorescences pollinated (and thus more seeds produced) produced significantly lighter seeds than under-pollinated plants. If seed number accurately reflects pollinator visitation rates, then variance in seed weight can be maintained as a consequence of fluctuating pollinator densities. At the level of the individual inflorescence, it was surprising to find that completely pollinated inflorescences had significantly heavier seeds than those in which only half of the flowers were pollinated. This finding contradicts the expectation based on a resource limitation argument where seed weight and seed number are negatively correlated. One possible explanation for this finding is that heavily-pollinated inflorescences represent a larger physiological sink for maternal resources.

Intraplant variation

One of the most striking results to emerge from this study was the temporal pattern of seed size within maternal plants: seeds formed early were larger than those formed later. This pattern has also been reported for other species (Salisbury 1958; Cavers and Steel 1984; Thompson and Pellmyr 1989; Winn 1991). Why this seasonal pattern exists is not obvious. Salisbury (1942) speculated that early seeds may be bigger because they have first access to a finite supply of maternal resources. Cavers and Steel (1984) suggested that seed weight variation would be greater in species with indeterminate growth since seeds would be produced over a long period and would consequently experience a greater range of micro-environmental conditions throughout the period of seed development. There is an additional consequence of indeterminate growth that may explain the temporal decline in seed weight. In *H. appendiculatum*, inflorescence size and flower size decrease through the season (Wolfe 1992) and those produced later in the season are borne on smaller stems (personal observation). It is known for some species that the size of structures is correlated with the size of supporting structures (Sinnott 1921), possibly due to less vascular tissue (Carlquist 1969). Consequently, as a result of the positive correlation between flower and seed size in *H. appendiculatum* (Wolfe 1992), smaller seeds will be produced on later inflorescences due to developmental constraints. In other words, regardless of the overall resource status of a maternal plant (which will be determined by pollination history and micro-environmental conditions), seed size will necessarily decline through the season as a result of a phenological change in plant architecture. Although the correlation between flower and seed size is not surprising given the developmental integrity between these two traits (Primack 1987), it may have important implications for the observed patterns of seed size. For example, owing to the phenotypic correlation between the two traits, a preference by pollinators for large flowers (Galen 1989; Schemske and Horvitz 1989; Young and Stanton 1990), could result in an increase in mean seed size.

Conclusions

In *H. appendiculatum* large seeds have a selective advantage over small seeds. Although emergence time and probability is independent of size, large seeds develop into larger seedlings which have higher survival. This was found to be true under both field and greenhouse conditions (Wolfe 1990). Yet, the apparent lack of additive genetic variation for seed size would suggest that this trait could not respond to directional selection that would result in an increase in the mean. Likewise, it does not seem that selection could operate to fix any one "optimal" seed size in *H. appendiculatum*, as would be predicted by various analytical models (Smith and Fretwell 1974; McGinley et al. 1987). I believe the most parsimonious explanation for the observed phenotypic variation in seed size is that individuals have only a limited ability to control seed size. Rather, phenotypic variance in seed size in *H. appendiculatum* is virtually guaranteed by the complex and dynamic nature of interactions of several ecological and genetic factors. Perhaps most importantly, temporally-related changes in flower size will result in smaller seeds being produced as the flowering season progresses. Adding further heterogeneity into this intrinsic pattern of change will be the less predictable nature of the pollination ecology which will influence the intensity of pollination, as well as type, and number of pollen donors siring seeds. Finally, randomness in the abiotic environment within a population will create differences in the mean seed size of individual maternal plants.

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