

Subindividual variation and genetic versus environmental effects on seed traits in a European *Aquilegia*

M.C. Castellanos, M. Medrano, and C.M. Herrera

Abstract: Repetitive plant parts such as seeds can be characterized at the within-individual level not only by average values but also by higher moments of their distributions, such as the standard deviation, skewness, and kurtosis. These parameters can potentially be adaptive and shaped by selection like any other individual trait, but tend to be understudied. We present an example of how within-individual variation might be studied. We used a diallel crossing design to investigate the sources of phenotypic variance of among- and within-individual variation in seed and germination traits of the endemic columbine *Aquilegia pyrenaica* subsp. *cazorlensis* (Heywood) Galiano & Rivas Martínez. Individual plants and even fruits have their characteristic mean, standard deviation, skewness, and kurtosis and therefore these parameters could potentially respond to selection. However, we only found evidence of genetic variance in mean within-fruit seed mass, mean time to seedling emergence, and number of seeds: these effects were attributable to maternal effects. Variance in higher moments of the within-fruit distributions in this species could instead reflect developmental instability or plastic responses to localized environmental conditions as fruits develop. Large sample sizes are required to make final conclusions in this case, but this study illustrates how the novel approach of considering high moments of intra-individual variation will enhance our view of plant trait evolution.

Key words: bio model, diallel, germination, maternal effects, seed mass.

Résumé : On peut caractériser les parties répétitives des plantes, telles que les graines, comme appartenant à un degré intra individuel non seulement pas leurs valeurs moyennes, mais aussi par leurs pics de distribution comme la déviation standard, l'asymétrie et la curtose. En général on accorde peu d'importance à ces paramètres qui peuvent tout de même représenter l'adaptation et être structurés par la sélection comme tout autre caractère individuel. Les auteurs présentent un exemple de la façon avec laquelle on peut étudier la variation intra individuelle. Ils utilisent un modèle basé sur le croisement de diallèles pour examiner les sources de variance phénotypique à partir de variations intra en entre individus, portant sur des caractères de germination, chez la plante endémique *Aquilegia pyrenaica* subsp. *cazorlensis* (Heywood) Galiano & Rivas Martínez (ancolie). Les plantes individuelles et même les fruits montrent une moyenne, une déviation standard, une asymétrie et une curtose caractéristiques et conséquemment ces paramètres pourraient réagir à la sélection. Cependant, les auteurs n'ont trouvé des preuves de variance génétique que pour la masse séminale du fruit individuel, la durée moyenne d'émergence de la plantule, et le nombre de graines; on attribue ces caractéristiques à des effets maternels. La variance des pics de la distribution au niveau du fruit chez cette espèce pourrait au contraire refléter une instabilité du développement ou des réactions plastiques à des conditions environnementales localisées, à mesure que se développent les fruits. Dans de tels cas, on doit compter sur des échantillonnages de grande taille pour tirer des conclusions finales, mais cette étude illustre comment une nouvelle approche considérant les pics de variation intra individuelle améliorera notre perception de l'évolution des caractères génétiques des plantes.

Mots-clés : biomodèle, diallele, germination, effets maternels, masse séminale.

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Introduction

Post-zygotic reproductive characters such as seed traits

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and germination time are intuitively expected to play a major role in plant reproductive success, because they are fundamental for successful establishment. Often, seed and germination traits are found to be related to plant fitness (Howe and Richter 1982; Stanton 1984; Biere 1991b; Simons and Johnston 2000) and subject to selection in natural conditions (Kalisz 1986, 1989; Gómez 2004; Donohue et al. 2005; Halpern 2005), although not always (Mazer 1987a; Winn 1988). Traditionally, seeds are described by the mean within individual plants, yet the variation around the mean could be an individual parameter as distinctive as the mean itself, and potentially with an adaptive value just as important (Roy 1959; Herrera 2008). Some aspects of the within-plant variation in seed features are probably the inevitable

consequence of architectural effects and space constraints (McGinley et al. 1990; Diggle 1995), but in other cases it may be an adaptive parameter in itself that confers some fitness advantage to the plants that exhibit it, over those that do not (e.g., Daws et al. 2007). This variability may constitute a bet-hedging strategy, whereby resources and (or) the risk of failure is either spread out or concentrated among reproductive units to maximize fitness, depending on the ecological conditions (Cohen 1966).

Examples of seed traits that are potentially variable within plants include size, germination behaviour, and dispersal capacity. How much individuals vary (among plants) in how variable their seeds and fruits are (within plants) may lead to differences in fitness by influencing the success of offspring recruitment. In wind-dispersed seeds, for instance, variation in seed mass could lead to differential dispersal distances, and thus, to variable seed shadows (Augsburger and Franson 1987). This can in turn affect the impact of density-dependent effects on fitness, such as predation and sibling competition, and the colonization of new habitats. However, empirical evidence for this is scarce, because seed shadows are very difficult to measure and follow up through cohort recruitment. There is more direct evidence for the effect of seed variation on seedling emergence time. For *Lychnis flos-cuculi* L. sown in experimental gaps in the field, Biere (1991b) found that a high variance in germination time seems to contribute to an increased survivorship of seedling progenies, and that individual differences in the variance of germination time within a season could explain survival differences among maternal genotypes.

Aside from the variation around the mean, even higher moments of the within-plant distributions of seed trait values (skewness and kurtosis) could be important descriptors of a plant's phenotype, particularly in plants that produce large numbers of seeds (McGinley et al. 1990, Simons 2007, Herrera 2008). The variance merely describes the amount of variation in an individual, but does not provide any information on the shape of the distribution of such variation, which can be highly variable among individuals (McGinley et al. 1990). The symmetry of the seed size distribution (measured by the skewness) and how peaked it is around the mean (measured by the kurtosis) are other parameters by which individuals could vary in ecologically meaningful ways. For example, a plant with a positively skewed distribution of germination times could benefit from the delayed germination of part of its seeds after an initial burst triggered by an external cue. Such skewness could reduce competition among seedlings or increase the chances of seed dispersal.

Like any other character, the parameters describing the within-plant distributions of seed traits (mean, variance, skewness, kurtosis) can have different sources of phenotypic variance and must have a genetic basis to respond to selection. In this paper we present a novel approach to study intra-individual variation in seed and germination traits. A first step to determine the potential for evolutionary change of such parameters is to partition their phenotypic variance into its possible components. Because of the complex nature of seed tissues, which combine maternal and paternal genes in varying proportions and are formed surrounded by an exclusively maternal environment, the relative importance of genetic versus environmental sources of pheno-

typic variation in seed and germination parameters are particularly interesting. The presence of maternal and paternal effects plus their interactions, and the extent to which variation has a genetic basis can inform us about the potential of such parameters for evolutionary change. We used a diallel crossing design to study the sources of phenotypic variation in mean, standard deviation, and higher moments of the within-plant distributions of seed traits in the endemic columbine *Aquilegia pyrenaica* subsp. *cazorlensis* (Heywood) Galiano & Rivas Martínez. Other studies have examined seed traits with diallel crossing designs in a number of plant species (e.g., Antonovics and Schmitt 1986; Schwaegerle and Levin 1990; Biere 1991a; Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Waser et al. 1995), but to our knowledge none have looked at the variance partitioning of parameters describing high moments of within-plant distributions of seed traits as we do here.

Methods

Study species

Aquilegia pyrenaica subsp. *cazorlensis* is a perennial herb endemic to a narrow area of the Sierras de Cazorla and El Pozo, Jaén province, in southeastern Spain. Populations grow between 1200 and 1950 m a.s.l. and are strictly restricted to damp, shady areas in rocky limestone outcrops, usually with sandy soils. *Aquilegia pyrenaica cazorlensis* populations appear to be isolated from each other by broad expanses of unsuitable habitat. Blooming occurs in early summer. The pale-blue flowers are protandrous, self-compatible, and have 4–7 free carpels that mature into independent follicles. Petals are extended to form long nectar spurs, as is usual in all species of the genus. Bumblebees are common visitors and the most important pollinators, although high fruit set occurs in the absence of insect visits. The fruits are follicles that open in late summer and release the seeds. No specific seed dispersal mechanism is present and most seeds fall to the ground within close proximity of the mother plant, which often results in dense patches of seedlings. Germination can occur shortly after dispersal and seeds do not appear to possess dormancy.

Crosses

For a full reciprocal diallel crossing design we used seven reproductive plants growing in pots in a pollinator-free greenhouse in Seville (Spain). At the time of the experiments, all plants were 2 years old. They had been grown from seeds collected in one *A. p. cazorlensis* population (Cerrada del Pintor, Sierra del Pozo), from plants randomly selected from the large number of individuals occurring at the locality. The relationships among our seven maternal plants are unknown.

We hand pollinated during 4–16 May 2005 (blooming in the greenhouse occurred earlier than in natural conditions). Each plant served both as a pollen donor and a pollen recipient in the reciprocal crossing design, and each cross was replicated twice when possible. Parental plants had 3–9 inflorescences, and the different crosses were interspersed among them. To prevent self-pollination, we emasculated flowers while still in bud before any anthers dehisced. We

also covered each flower's styles with a small piece of plastic straw to prevent any pollen from reaching the stigmas. We removed the piece of straw only briefly at the moment of hand pollination, two or three days after anthesis, and kept it in place for several days until the stigmas had dried out as part of fruit development. To pollinate, we used one or more freshly dehisced anthers from the donor plant. We held the anther with forceps and rubbed it against the receptive stigmas of the recipient flower until they looked saturated with pollen. Receptive stigmas in this species are easy to recognize, because they curl up and appear sticky.

We define a "fruit" as the set of follicles originating from a flower, irrespective of their number. Fruits from crosses were collected just before seeds were to be released, and stored separately in paper envelopes at room temperature. We planted a subsample of 15 seeds (or less when seed set was lower) from each fruit on 30 July, after all experimental fruits had been collected. Five replicates of three seeds were planted in small starting pots using a 4:1 mix (v/v) of peat and vermiculite, and kept outdoors in a shaded area with no temperature control. We checked for seedling emergence every day for the first 2 weeks and then every other day for 5 more weeks, and recorded the date as the time when cotyledons were first visible and had started to spread apart. We restricted our analyses to those seedlings that emerged within two months of sowing, after which germination was very rare. Overall emergence success was 40.2% of 1361 seeds sown.

Traits and analysis

For each fruit, we considered the number of seeds produced, and the mass and time to seedling emergence of individual seeds. Given the variable number of follicles, seed production was standardized to a per-follicle basis by dividing the total number of seeds in the fruit by the number of follicles that set at least one seed. From each fruit, we randomly sampled 10 seeds and then weighed them individually on a digital balance to the nearest 0.01 mg. For each fruit, we calculated the mean, standard deviation (SD), skewness and kurtosis of individual seed mass, and time to seedling emergence. In total then, we analyzed nine parameters, all of which had the fruit as the statistical unit. Final sample sizes were lower than the maximum possible of 84 fruits in the crossing design because nine replicates were not possible and a few more fruits rendered no data. Low germination success in a few crosses further reduced sample sizes for the within-fruit variability parameters in time to seedling emergence.

We partitioned the sources of phenotypic variance for each distribution parameter using the program quercus (Shaw and Shaw 1994), which uses restricted maximum likelihood and therefore allows for the few blanks in our data set. The analysis is based on the "bio model" by Cockerham and Weir (1977), which in a full diallel design allows for the partitioning of phenotypic variance V_P among individuals into six components: V_A , additive genetic variance, attributable to the effect of nuclear alleles expressed in the seed; V_D , dominance variance, accounting for the interaction of alleles within nuclear loci; V_{mat} , maternal variance, accounting for variance that is not additive and is specific to maternal function, such as cytoplasmic factors;

Table 1. Sample sizes (number of full-sib families, i.e., fruits) and ranges of family values for seven diallel parameters studied in *Aquilegia pyrenaica cazorlensis*.

Trait	<i>N</i>	Range
Seeds per carpel	72	2 – 21.8
Seed mass (mg)		
Mean	74	0.53 – 0.99
SD	74	0.029 – 0.147
Skewness	74	-1.78 – 1.04
Kurtosis	74	-1.91 – 4.70
Time to seedling emergence (days)		
Mean	73	14.0 – 47.0
SD	68	1.15 – 13.45
Skewness	63	-1.34 – 3.02
Kurtosis	59	-4.07 – 9.93

V_{pat} , paternal variance analogous to the maternal variance; V_K , higher interactions, such as could be caused by extranuclear parental interactions that are not part of the dominance variance; and V_E , environmental variance expressed as variance within cells of the diallel. The model assumes negligible epistasis and genotype-by-environment interactions. Self crosses were excluded from this analysis because potential inbreeding effects are not accounted for by the model. Because the analysis does not distinguish between replicates within a single fruit and replicates of a combination of parents from different fruits on the same maternal plant, we did not perform the tests on individual seed data, but rather on variables at the whole fruit level, as noted earlier.

No transformations were necessary for most variables, as they were normally distributed, except for the kurtosis of both seed mass and time to seedling emergence, which were ln-transformed. For each variable we first obtained estimates of the six components of variance for the full model. Components estimated as negative were constrained to zero in subsequent analyses. We then used likelihood-ratio tests to evaluate if variance components in each model were statistically different from zero (Shaw 1987), with the exception of the V_E component, whose significance was not statistically tested.

Because all the analyses were run for each parameter independently, our interpretation of results could be confounded by correlations among them. To identify potential relationships, we evaluated Pearson correlations for all variable pairs. To detect potential effects of inbreeding, which could also influence the evolutionary fate of seed characters, we tested for overall differences between self and outcrossed progenies in all variables.

Results

We found ample variation among families produced by the diallel crosses in all nine variables (Table 1), including the higher moments of the distribution of seed traits, i.e., the SD, skewness and kurtosis of seed mass, and time to emergence of seedlings from the same fruit. This implies that individual fruits pollinated by the same sire vary in how heterogeneous their repetitive products are. The variability around the mean, and even the third and fourth moments of seed trait distributions, can thus properly be

Table 2. Percentage contribution of six components to the total phenotypic variance of seed trait distribution parameters in *Aquilegia pyrenaica cazorlensis*, from diallel crosses.

Phenotypic parameter	V_A	V_D	V_{mat}	V_{pat}	V_K	V_E
Seeds per carpel	–	15.4	47.7***	0.2	–	36.7
Seed mass						
Mean	–	–	59.0***	1.4	–	39.6
SD	16.8	–	–	0.2	–	83.0
Skewness	–	–	5.8	1.6	6.0	86.6
Kurtosis	20.7	–	–	–	–	79.3
Time to seedling emergence						
Mean	29.5	–	25.3*	1.2	–	44.0
SD	18.0	–	–	–	–	82.0
Skewness	–	–	9.2	4.2	–	86.6
Kurtosis	1.6	–	–	–	–	98.4

Note: *, $P < 0.05$; ***, $P < 0.001$; dashes (–) indicate components estimated as negative in initial analyses that were constrained to zero for the final model. V_E was not tested for significance.

regarded as descriptors of a fruit. Within-individual variation in time to seedling emergence could be even higher than reflected here, because our germination trials were started synchronously even though fruit ripening was not necessarily so.

The mean values for seed mass, time to seedling emergence, and the number of seeds per follicle all showed strong and significant maternal effects (V_{mat}) that explain most of the phenotypic variance (Table 2). In contrast, the phenotypic variances of higher moments of the within-fruit distributions of seed mass and time to seedling emergence do not appear to have a genetic basis. For these six variables, the only important source of phenotypic variance in the analysis was environmental (residual, V_E) variance. Variance specific to paternal function (V_{pat}) was very low in all cases. Additive genetic variance (V_A) was never significant even though in several cases the estimate seemed substantial (Table 2). Note that our distribution parameter estimates reflect only the component of within-plant variation that occurs at the within-fruit level. Although this component accounts for most within-plant variance (>94% and >99% for seed weight and time to seedling emergence, respectively) and exceeds by far the component due to differences among fruits of the same plant, the possibility cannot be ruled out that slightly different phenotypic variance partitions would be obtained if variation at the whole-plant level (i.e., within-fruit plus among-fruit components) were considered.

Correlation analysis showed that only a few pairs of variables were significantly correlated (Table 3). No trade-off seems to exist between the number of seeds per carpel and the mean seed weight in a fruit, as the two variables are, if anything, positively correlated ($r = 0.22$, $P = 0.06$). Seed mass skewness and kurtosis were negatively correlated, but further inspection revealed that the relationship between the two variables was actually more complex than linear: the kurtosis was higher for both small and large values of skewness. This implies that within-fruit seed mass distributions that are asymmetrical in either direction are also more peaked than symmetrical distributions. The same was the case for the skewness and kurtosis of the time to seedling emergence. These concave relationships might reflect a bio-

logical phenomenon, but we have limited information so far to understand its causes. The kurtosis of seed mass was also positively correlated with the SD of time to seedling emergence, i.e., seed cohorts with a more peaked distribution of seed weights around the mean also have a more variable time to germination. Finally, the mean time to seedling emergence was negatively correlated with the skewness of the same trait.

When comparing self versus outcrossed progenies from the diallel experiment, we found a potential effect of inbreeding only on seed mass. Outcrossed fruits produced on average heavier seeds (0.76 mg, $N = 74$) than those resulting from self pollinations (0.68 mg, $N = 12$; $t = 2.76$, $P = 0.007$).

Discussion

Maternal effects in traditional mean trait values

Maternal effects dominate the variation of mean seed traits in our diallel crosses. For seed number per carpel and mean seed size, respectively, 59% and 48% of the phenotypic variance was explained by maternal effects. We also found a significant maternal effect on the time to seedling emergence (about 25% of the V_P), though not as high as for seed mass. Similarly strong maternal effects are commonly reported for other species in seed size (Antonovics and Schmitt 1986; Mazer 1987b; Schwaegerle and Levin 1990; Biere 1991a; Platenkamp and Shaw 1993; Montalvo and Shaw 1994; Schmid and Dolt 1994; Waser et al. 1995; Wolfe 1995; Helenurm and Schaal 1996; Byers et al. 1997; Lipow and Wyatt 1999), seed numbers (Mazer 1987b; Waser et al. 1995; Good-Avila and Stephenson 2003), and time to germination (Biere 1991a; Platenkamp and Shaw 1993; Schmid and Dolt 1994; Helenurm and Schaal 1996), including the columbine *Aquilegia caerulea* (Montalvo and Shaw 1994). The importance of maternal effects in these traits is hardly surprising, because seeds develop surrounded and provisioned by maternal tissue and, in addition, the genetic contribution of mothers to seeds is higher compared to that of fathers. For instance, seed position and environmental effects during fruit development (e.g., maternal photoperiod, soil moisture, etc.) as well as genetic maternal factors

Table 3. Phenotypic correlations among seven seed trait distribution parameters studied in *Aquilegia pyrenaica cazorlensis* diallel crosses.

	Seed mass				Time to seedling emergence			
	Mean	SD	Skewness	Kurtosis	Mean	SD	Skewness	Kurtosis
Seeds per carpel	0.22 ^a	-0.07	0.08	-0.01	0.19	0.03	-0.05	-0.00
Mean seed mass		-0.15	-0.00	-0.04	0.18	-0.09	-0.02	-0.08
SD of seed mass			-0.20	-0.02	0.00	-0.14	0.17	0.19
Skewness of seed mass				-0.55***	0.12	-0.07	0.20	-0.09
Kurtosis of seed mass					-0.09	0.28**	0.12	0.15
Mean time to seedling emergence						0.03	-0.29*	-0.24
SD of time to seedling emergence							-0.03	-0.01
Skewness of t. to seedling emergence								0.59***

Note: Sample sizes are given in Table 1. SD, standard deviation; ^a, $P = 0.06$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

affect when a seed germinates (Baskin and Baskin 1998). Consistently, paternal effects are much less common in comparison.

Our analysis does not directly distinguish between genetic and environmentally induced maternal effects. However, the homogeneous environment and common growth history of all plants used for the crosses suggest that at least a large part of the effect has a genetic basis. We cannot rule out that localized environmental variation in greenhouse conditions might have affected mother plants differently, but it seems unlikely in a small group of plants of the same age growing within 2 m². Structural differences among maternal plants are more likely to have caused maternal environmental effects; for instance, we found a positive but weak correlation between mean number of seeds per carpel and mean seed weight, which suggests a size effect: fruits with many seeds also produce heavier seeds, possibly a maternal effect where larger mothers produce larger fruits on the whole. Architectural effects common in many plants, such as the position of the fruit in the inflorescence (Obeso 1993; Wolfe 1995), are also unlikely because we chose flowers haphazardly for pollination in different inflorescences of each plant. Thus our experimental conditions suggest that maternal environmental differences were weak, and therefore, the presence of genetic maternal variation in these parameters means that they could respond to selection through the mothers. On the other hand, the very small values of V_{pat} for all parameters (Table 2) indicate that there were no differences among pollen donors and thus no detectable potential for evolution through paternal effects, in agreement with similar studies (e.g., Schwaegerle and Levin 1990; Montalvo and Shaw 1994; Lipow and Wyatt 1999).

All other components of V_p were low for seed numbers and mean seed size and time to seedling emergence, except for V_E , the residual variance, which was substantial in all three cases. Here, V_E reflects differences among fruits within plants. This could imply a high sensitivity to microenvironmental variation that affected fruits or germinating seeds in a way independent of the maternal plant. Simons and Johnston (2006), for instance, found that the timing of seed germination was affected by small environmental variation even inside a growth chamber, and our less controlled germinating conditions could be reflected in our results for *A. p. cazorlensis*. Such variation could still have a genetic basis, a possibility that we tested by including in our analy-

ses the SD, skewness, and kurtosis of the within-fruit distribution of seeds traits.

Subindividual variability and higher moments of trait distributions

Our approach of considering several parameters of the within-fruit distribution of seed traits in a diallel crossing design provides a relatively unexplored view of plant features. It is an important aspect, because within-plant variability in seed traits can be adaptive if it translates into higher relative fitness during the lifetime of a plant than would be obtained if the plant produced homogeneous seeds. For instance, a bet-hedging strategy in seed germination can confer a plant the chance to produce at least some viable progeny in environments where ideal germination conditions are unpredictable (Cohen 1966). The ideal conditions might depend on variation in external factors if seeds are dispersed away to variable locations, on varying weather conditions within and among reproductive seasons, or on the density of competing siblings. For intra-individual variation in seeds to evolve, selection has to act on the variance of the trait, provided of course that the variation is (or was) heritable. In our study species, we found individual variation in within-fruit SD, skewness and kurtosis of seed size and time to seedling emergence. Thus, these distribution parameters can and should be treated as individual characteristics like any other.

Our crosses, however, suggest that the phenotypic variance in all such parameters has very low genetic, heritable variance as most of it cannot be assigned to genetic causes (high V_E). This is particularly interesting given that the average values of the same traits are under strong maternal influence. Instead, the within fruit variation seems to be largely independent of the genotype or parental effects. These results, however, should be taken cautiously since sample sizes were small. To detect genetic variance in higher order parameters, our statistical power can be small. Nonetheless, indirect evidence from other plant species is consistent with our results. For example, studies that have partitioned the sources of variance in seed weight have often found that most variance occurs within individual plants (e.g., Thompson 1984; Michaels et al. 1988; Obeso 1993). Even studies that report strong maternal effects on seed size or time to germination usually also find high V_E , which in many cases reflects variation between seeds or fruits among cross repli-

cates (see e.g., Antonovics and Schmitt 1986; Platenkamp and Shaw 1993; Byers et al. 1997), a level of intra-individual variation with no genetic basis. All this suggests that the parameters describing the within-plant distribution of seed traits could reflect either developmental instability or plastic responses to small differences in localized environmental conditions as the fruits develop. This is further supported by the substantial range of within-fruit variation in the skewness, kurtosis, and SD we found for *A. p. cazorlensis* traits.

If optimal levels of within-plant variation in seed weight or time to seedling emergence exist in *Aquilegia* plants, mothers seem to have little control over such variation even under relatively homogeneous growing surroundings, as seems also to be the case for other species like *Prunella vulgaris* (Winn 1991). For instance, because time to seedling emergence is unrelated to seed size in *A. p. cazorlensis*, mother plants would have to rely on another mechanism for controlling when their offspring germinate. Perhaps there is no such mechanism available in this species, although it is also possible that, as suggested by Simons and Johnston (2006), localized "microplasticity" in itself might operate as a mechanism for maintaining within-individual variability. Alternatively of course, within-plant variation in seed traits might be neutral for *A. p. cazorlensis* plants without any fitness consequences.

Selection on seed characteristics in an endemic Mediterranean plant

In addition to the low number of plants used in our design, three factors could limit our ability to make inferences about the response to selection in the seed-related plant parameters we studied. First, as discussed above, diallel crossing experiments do not, per se, properly allow for the separation of genetic and nongenetic variance components (Shaw and Waser 1994); even purely environmental effects can be transmitted over generations in plants (Miao et al. 1991). In our crosses, however, the common growth history of parents suggests that the maternal effects can be attributed to genetic variation. Second, the capacity for inferences on trait evolution from greenhouse studies is generally limited. Usually, heritabilities estimated in controlled environments are over-estimated, because the effects of the environment are neutralized with respect to natural field conditions (Schwaegerle and Levin 1990). In the current study, environmental variance was strong for most parameters in this study, even in the greenhouse, so our results do not ignore the importance of environmental variation. The actual extent of environmental variance and other factors that might affect the response to selection, such as competition, could still be higher in natural conditions and therefore be underestimated in our results. Finally, even though we eliminated self crosses from the experimental design, it is still possible that some of the seven parent plants were closely related and that inbreeding explains some of the variance in seed traits. Inbreeding is not considered in the model used, but it is likely common in the small populations of the study species.

In spite of these potential caveats, and the low narrow-sense heritabilities detected for all parameters in this study, our results do not rule out the potential for selection to act

on seed number per carpel, mean seed size, and mean time to seedling emergence in *A. p. cazorlensis* plants. Strong genetic maternal effects for these three parameters suggest that they are at least partly heritable, and evolutionary change would be possible as indirect responses through selection on maternal genotypes (Biere 1991b; Byers et al. 1997; Wolf et al. 1998). For this particular species, selection on seed properties might be stronger for the local persistence of the populations rather than for long-distance seed dispersal, as has been suggested for other endemics (Lavergne et al. 2004). *Aquilegia p. cazorlensis* populations are restricted to shady and humid conditions that are only available at very few, disconnected spots that are surrounded by dry inhospitable areas. Under these conditions, dispersal distances greater than a few meters from the maternal plant might be fatal, potentially favouring selection for heavy seeds. However, although our greenhouse study was able to detect genetic variation expressed as differences in maternal phenotypes, in natural populations there might be limited opportunity for selection because (i) as discussed above, environmental variance might overwhelm maternal genetic variation; (ii) populations are small and seemingly isolated and negative genetic effects due to inbreeding are expressed at least by a reduced seed size; and (iii) maturing fruits are exposed to high levels of herbivory by both insects and mammals, which might further reduce the variance exposed to selection.

For this particular species, our results suggest that plants have little control over crop characteristics. For many other plants, within-individual variation might be playing different roles. The study of within-plant distribution of phenotypic variance provides a different and important perspective of the evolution of plant characters that enhances the traditional mean-centered approach.

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